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In vitro culture of the pejibaye palm (Bactris gasipaes H.B.K.)

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by

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GENERAL INTRODUCTION

Description of the Pejibaye Palm

Before proceeding to describe the nature of the study undertaken here, and before reviewing the literature relating to that study, some general information will be provided about the taxonomic, agronomic, chemical, and socio-economic aspects of the pejibaye palm, Bactris gasipaes H.B.K., as well as its natural history.

Taxonomy

The pejibaye or peach palm is a monocotyledon belonging to the family Palmae, the sub-tribe Bactroideae, and the genus Bactris (Tomlinson, 1961). Most of the species were identified between the late 19th century and the middle of this century. Although many of the species originally classified under the genus Guillielma are now classified under the genus Bactris, some controversy still exists about the distinction between the two genera, and hence about the classification of species under either genus. At present, the genus Bactris contains 187 species, 14 of which had been classified under the genus Guillielma at one time or another (Mora Urpí and Clement, 1981).

The pejibaye palm has a very straight, slender trunk which, once the plant has reached maturity, has a diameter of only about 15 cm (Popenoe and Jiménez, 1921). Its root system consists of two portions. One is shallow and extends up to 5 m from the trunk, the other grows up to 2 m into the ground (Vandermeer, 1977). Because of a system of associated vesicular-arbuscular

mycorrhizae, the root system is able to absorb phosphorus in very acidic soils (Janos, 1977). Like the date palm, Phoenix dactylifera, the pejibaye palm produces offshoots which arise from its rhizome. If the dominant shoot is pruned properly, offshoots continue emerging over an unlimited number of years (Mora-Urpí et al., 1984; Popenoe and Jiménez, 1921). The number of basal offshoots depends primarily on the age of the tree, since offshoot production tends to decrease with age (Mora-Urpí et al., 1984). The average lifetime of the pejibaye palm is about 50 years. Old plants may be as tall as 20 m, with large spines (up to 6 cm) on the internodes of most species (Popenoe and Jiménez, 1921). Such spines represent not only an adaptation for direct physical protection, but also for indirect protection: the spines improve drainage of rain water away from the stem and discourage the establishment of epiphytes on the stem, thus making it less susceptible to insect and fungal damage, given that insects and fungi are often associated with the root system of epiphytes (Mora-Urpí, 1983). No dwarf species have been found in nature so far.

Mature palms have feathery leaves, up to 3.6 m in length, and each leaf has a bud in its axil. Buds that are located at the base of the stem will develop into offshoots. Buds that are located in the axil of an aerial leaf develop into inflorescences in most cases (Popenoe and Jiménez, 1921). On very rare occasions aerial buds develop into offshoots (Mora-Urpí, 1983).

The pejibaye palm is self-incompatible in most cases (Mora-Urpí et al., 1984), and as a result outcrossing is promoted, a process which maintains the large genetic variation of the species. Such a pronounced genetic variability from palm to palm affects all traits of commercial importance, including the chemical composition of the fruits (Arkcoll and Aguiar, 1984). Each inflorescence

is contained in a large spathe which bursts open at the time of anthesis. Inflorescences have thousands of staminate flowers, but only hundreds of pistillate flowers (Calzada et al., 1977; Mora-Urpí and Solís, 1980). The occurrence of parthenocarpy is fairly common in cases where pollination is deficient, but racemes with parthenogenic fruits contain relatively few fruits (Mora-Urpí, 1983), which frequently abort before maturity (Johannessen, 1966a). Pollination is carried out by insects (mostly by Deremolus palmarum Champ. of the order Coleoptera), wind, and gravity (Mora-Urpí and Solís, 1980). In the wild, the palms start producing fruit on four- to five-year-old plants, whereas under cultivation, fruiting may be observed as early as two years after seed germination, under ideal rainfall and sunlight conditions (Mora-Urpí, 1983).

The fruits grow on racemes which are attached just below the aerial leaves. A plant produces between 4 and 12 racemes per year (Johannessen, 1967), each of which carries an average of 96 fruits (Arkcoll and Aguiar, 1984). Much higher yields have been observed, such as 300 fruits per raceme (Balick, 1979) or even 1,000 fruits per raceme (Arkcoll and Aguiar, 1985), but these are not typical. Flowering is determined mainly by the alternating dry and rainy seasons, but also by the nutritional status of the plant. Under conditions of ample rainfall fruit production occurs twice a year (Johannessen, 1966a; Mora-Urpí, 1983). The fruits are peach-sized (hence the name peach palm) and shaped like a persimmon, with a green or yellow skin and orange mesocarp, and they usually contain a single seed. The exceptions to this are the cases when a fruit contains two fused seeds, which occurred in about 5 % of the cases in the mixed seed lot imported from Costa Rica for the research presented here. Johannessen (1966a) reported fused seeds in 0.1 % of all cases. The mesocarp is fibrous and mealy, especially when the oil

content is relatively low. It has a pleasant, chestnut-like taste when cooked (Johannessen, 1967; Popenoe and Jiménez, 1921).

An almost endless number of names are used for Bactris gasipaes H.B.K., depending on the region and social group in which they are used. A few examples are listed below (Popenoe and Jiménez, 1921; Seibert, 1950; National Research Council, 1975; Mora-Urpí, 1983):

Popular name	Country
chontaduro, gachipaes, cachipay	Colombia
inchuay, pijuaio, nieje	Perú
pejibaye, pixbae, piriguaio	Costa Rica
pupunha, tapiré	Brasil
peach palm	Trinidad
paripou	French Guiana

Natural history

The range of the pejibaye palm extends from Bolivia (17°S) to Honduras (17°N). It is native to the tropical rainforest and thrives under conditions of heavy rains (2,000 to 6,000 mm per year). The palm is well adapted to poor, heavy soils, although it requires good drainage. In the wild it grows in low natural densities from about sea level to an altitude of 400 m, an environment which in the humid tropics remain at temperatures between 24 and 28°C (Mora-Urpí, 1983; Popenoe and Jiménez, 1921). However, some species of the genus Bactris grow in

southern Florida and have survived periods of mild frost without being damaged (Popenoe, 1977).

The pejibaye palm cannot be traced to a single location of evolutionary origin; in fact, it is believed that several wild types of the pejibaye palm evolved all throughout the Americas, within their range of geographical distribution (Mora-Urpí, 1983). Today's commercial Bactris gasipaes H.B.K. is thought to be of hybrid origin, because it is always associated with centers of human activity and has never been found in the wild. It has, however, been cultivated since pre-Columbian times by Amerindians, and it is they, in fact, who have played an important role in bringing about the hybridization and distribution of the pejibaye palm since well before 2,000 years ago. Hybridization centers of the pejibaye palm can be traced to large Indian population centers, such as Belém in Brasil, and Iquitos in Perú (Mora-Urpí, 1979, 1983). The fruits of wild types tend to be quite small in comparison with the hybrid pejibaye fruits (Mora-Urpí, 1981).

The wild types of the Bactris and Guillielma genera can be grouped into two major categories, according to their characteristics and their geographic distribution. Species growing west and northwest of the Andes, ranging into Central America, tend to have hard, very spiny stems, and more offshoots. Those native to areas east of the Andes and extending south to the Amazon Basin, have softer stems with fewer offshoots, and a corkier, more resistant epidermis, which may be an adaptation that counterbalances the reduced number of spines (Mora-Urpí, 1983).

Agronomic traits

As already mentioned above, the pejibaye palm is well adapted to heavy soils, but requires good drainage. It grows well on soils with low fertility levels, but is very responsive to the addition of fertilizers. The shallow portion of its root system is very susceptible to weed competition, especially when nitrogen is in scarce supply, and weeds should therefore be kept under control (Mora-Urpí et al., 1984). Under optimal fertilizer and ground treatments, the pejibaye palm may more than double its yields, compared with its average performance on good soils under non-fertilized conditions (Arkcoll and Aguiar, 1984).

The primary method of propagation of the pejibaye palm has been the germination of seeds and the subsequent transfer of seedlings into the field (Mora-Urpí, 1983). Asexual methods of propagation, be they through the use of basal offshoots or the in vitro regeneration of plantlets from somatic tissue, have yielded inconsistent results and have therefore not yet been utilized on a commercial scale (an in-depth discussion of asexual methods of propagation will follow, as this is the focus of the research described in this thesis).

Seeds are germinated either by placing them in flats containing soil, or by disinfesting them, moistening them, and placing them in sealed polyethylene bags. This latter method of germination is the fastest (about 2 months) and most reliable (more than 90 % germination rate). Seedlings are transplanted 3 to 6 months after germination. Spacing between the seedlings in the field depends on the use intended for the palm crop: if it is established for the production of "heart-of-palm", spacing need not exceed 1.5 m; if, on the other hand, fruit production is desired, spacing should be at least 5 m (Mora-Urpí, 1983). Fruit

yields of the pejibaye palm range quite widely, due to the heterogeneity of the species, but under favorable conditions yields of 25 metric tons per hectare are common (Mora-Urpí et al., 1984). Such yields far exceed the yields of any cereal crops grown in the same geographical area, including corn (Hunter, 1969).

Pejibaye production is not, at present, seriously threatened by any disease (Clement and Mora-Urpí, 1982), unlike the coconut and oil palms, in which the heartrot and lethal yellowing diseases have caused substantial economic damage (Attias et al., 1987). Pests and diseases of the pejibaye palm include various beetles which act as larva and nematode vectors and affect mostly young plants and the rhizome, and six kinds of fungi of the genus Monilia which affect the fruits and leaves (Mora-Urpí, 1983).

Chemical characteristics of the fruit

The National Research Council (1975) stated that the pejibaye fruit is "probably the most balanced of all tropical foods, [containing]... carbohydrates, protein, oil, minerals, and vitamins...[It] has twice the protein content of banana and can provide more carbohydrate and protein per hectare than maize." It contains moderately small amounts of 7 of the 8 essential amino acids not synthesized by humans (Cooz, 1984, in Tracy, 1985), the exception being tryptophan. Its lysine content is comparable to that of the "Opaque 2" high lysine mutants of corn (Mertz et al., 1964, in Tracy, 1985). It contains high levels of vitamin A and C, oil, and prime quality protein. Carbohydrates are present mostly in the form of starches (Mora-Urpí et al., 1984).

The overall composition of the oils contained in the pejibaye mesocarp and endocarp is similar to that of the African oil palm, although the pejibaye

palm has, on the average, a lower oil content per weight than the oil palm (Zapata, 1971). As in other palms, the oil composition of the fruit mesocarp in the pejibaye palm differs considerably from that of the fruit endocarp (Hammond et al., 1982). The oil of the pejibaye fruit mesocarp contains a high percentage of unsaturated fats compared to coconut oil and African oil-palm oil (Hammond et al., 1982; Zumbado and Murillo, 1984), and this may influence the pejibaye industry positively in the future due to the growing awareness among consumers about the harmful effects of saturated fats.

There is, however, one major drawback to this remarkable fruit, namely the presence of a proteolytic enzyme inhibitor, possibly oxalic acid, which affects monogastric animals such as humans, swine, and chickens. In at least one study, chicks that were fed raw pejibaye meal died within several hours. This proteolytic enzyme inhibitor, however, degrades very quickly upon exposure to heat. Autoclaving, boiling, roasting, or frying completely eliminate the problem for humans and other monogastric animals (Arkcoll and Aguiar, 1984; Murillo et al., 1983).

Uses for the entire palm tree

The list of possible uses for various components of the pejibaye palm is difficult to exhaust. Below are only a few examples of such uses (Popenoe and Jiménez, 1921; Mora-Urpí et al., 1984).

Fruit As a vegetable when green; boiled or "French fried" when ripe; processed into flour for use in bread in tortillas; processed for oil extraction and for use as animal feed; or for the production of alcohol or wine.

Palm Heart Fresh, in the form of a salad, toasted as cereal, or ground very finely to make drinks.

Inflorescences and leaves Inflorescences may be pickled in vinegar or oil with the addition of condiments; the pollen may be used in apiculture; some non-spiny pejibaye plants have variegated leaves and may serve as ornamental plants; mature leaves serve as roofing material.

Stem The main stem of mature plants is extremely hard and has been used for centuries to make bows and arrows, fishing rods, and other tools. The stems are black in color and extremely durable, and they are used in construction as ornamental wood or part of the building structure. Cellulose for cellophane and rayon, and even liquor may be obtained from the softer parts of the stem. A brief but lively account of one of the many uses of the stem of the pejibaye palm, i.e., for making parrot cages, can be found in Alfred Russel Wallace's book Palm Trees of the Amazon (1971).

Currently, the bulk of the commercial use of the pejibaye lies in the consumption of the pejibaye fruit by humans, and in the harvest of the heart of palm (Balick, 1976; Mora-Urpí et al., 1984). Use of pejibaye fruit meal as animal feed is so far restricted to the feeding of swine, who like the seeds especially (Johannessen, 1966a). Goals for the future use of the pejibaye fruit are set on a large, commercial scale, and include the extraction of its oil for human consumption, the production of animal feed from the fruit meal remaining after oil extraction, and the use of pejibaye flour in large-scale bread and tortilla production. Since the pejibaye meal and corn have comparable nutrition levels, the use of pejibaye meal as animal feed would reduce current corn imports and

improve the trade balance of the pejibaye-producing countries (Murillo et al., 1983; Zumbado and Murillo, 1984; Tracy, 1986).

Problems

In order to provide a complete picture of the full potential of the pejibaye palm as a commercial crop, not only the many advantages should be underscored, but also the disadvantageous characteristics of the crop. There are several problems of differing degree of importance. The most notable obstacle to the production of the pejibaye palm on a commercial scale is the large genetic variability within the species. This variability affects every phenotypic expression of the palm, including its fruit production and the chemical composition of the fruit (Mora-Urpí and Clement, 1981). This is why research on methods of asexual propagation for the pejibaye palm are so important.

The sharp, long spines on most adult pejibaye palms make it dangerous and awkward to harvest the fruit bunches (Hunter, 1969). Increased propagation of spineless varieties will alleviate this difficulty (Balick, 1976).

Another problem is the presence of the proteolytic enzyme inhibitor (Murillo et al., 1983). Although it has been well established that this compound degrades easily and to safe levels, if not completely, with the application of heat, more research is needed to determine its exact chemical nature and the lowest temperature possible at which it degrades.

Fruit production in the pejibaye palm is seasonal (Mora-Urpí, 1983), which means that farmers cannot rely solely on the pejibaye palm for their year-round income. Some research on intercropping in pejibaye groves has been

carried out, but more work in this area is needed (National Research Council, 1975; Tracy, 1985).

The pejibaye fruit has a tendency to spoil rather quickly once it is harvested, unless it is dried to an 8 to 12 % moisture content (Johannessen, 1966a; National Research Council, 1975; Tracy, 1985). More research and feasibility studies are needed to determine the best ways to store or treat the fruit after harvesting.

Another category of challenges includes those dealing with the human and socio-economic aspects of large-scale pejibaye production, such as developing a mechanized method of fruit processing, developing a strong market basis for the crop so that market prices improve over the currently inadequate levels, and conducting a public relations campaign which will promote the image of the palm and thus motivate its use in previously unknown ways (Tracy, 1985).

Finally, there is a global lack of awareness about the pejibaye palm which needs to be overcome. The species is known and utilized fairly widely in Costa Rica, but its importance, and even existence, is ignored in many other regions of Latin America (Hunter, 1969). Due to the enormous potential of the pejibaye palm, a crop that is ideally suited to the poor and easily eroded soils of the humid tropics, it is the responsibility of all researchers involved in studying it to promote widespread knowledge about it.

The second half of this literature review will summarize the research that has been carried out on the <u>in vitro</u> propagation of not only the pejibaye palm, but also the date palm, the African oil palm, and the coconut palm. It is necessary to review the information concerning these latter three species as well because of the phylogenetic and commercial similarities between them and the

pejibaye palm, and also because more tissue culture research has been conducted on them, much of which may be directly pertinent to the in vitro culture of the pejibaye palm.

Asexual Propagation

Traditionally, the pejibaye palm has been propagated by seed germination and subsequent transfer of seedlings to the field. This method is very reliable and easy to carry out (Mora-Urpí, 1980), but it does not provide an answer to the problem of needing to control genetic segregation. As already mentioned above, the pejibaye palm, like the date palm, coconut palm, and African oil palm, is extremely heterozygous as a species, and this heterozygosity, together with its long vegetative cycle (it takes at least two years before fruit production onset), renders conventional breeding methods very slow and inefficient.

Far better ways to retain the desirable characteristics of elite pejibaye palms, selected from a given stand, are to separate offshoots from the mother plant and propagate those, or to clone selected palms via tissue culture. Some research has been carried out on the former method of asexual propagation (Gonzales and Dominguez, 1977, cited in Sattler, 1985a; Arias, 1979; Blaak, 1980; Sattler, 1985), but this method is not very efficient, since thus far the highest survival rates of offshoots separated from their mother stems and cultivated under optimum field conditions have not exceeded 63.3±20.8 % (Centro Agronómico Tropical de Investigación y Enseñanza, 1986). The number of basal offshoots produced is determined by the age of the tree and by its genotype. Hence, the

average number of offshoots produced varies widely from tree to tree. If the average offshoot production could be increased in a consistent manner, perhaps the low yields of direct offshoot propagation could be partially offset. Positive results in this direction have been obtained through the application of the morphactin flurenol (Arias, 1979) and through the destruction of the apical meristem by means of a corkborer (Blaak, 1980). Date palm offshoots have commonly been used as propagules for vegetative propagation, and there has been no mention that this method is unreliable (Sharma et al., 1984; Tisserat, 1984c). There is hope then, that further research on pejibaye offshoot propagation will lead to more promising results.

The most efficient alternative for the asexual propagation of the pejibaye palm, once a method for it is refined, would be in vitro cloning. At present, only the African oil palm is propagated on a commercial scale via tissue culture (Paranjothy, 1986), although somatic embryogenesis and plantlet regeneration has been obtained in all four palm species reviewed here (Tisserat, 1979a; Paranjothy, 1986; Valverde et al., 1987). Tissue culture propagation of palms could also be used in conjunction with traditional plant breeding programs, for example if dihaploid plants were generated via anther culture and then used to produce hybrids. Conversely, F_1 hybrids or any other palms already selected in breeding can theoretically be cloned and reproduced in infinite numbers.

Compared to dicotyledons, the tissue culture propagation of monocotyledons, and notoriously that of palms, is rather erratic and difficult, the reasons for which are not entirely clear (Hunault, 1979). Not surprisingly, plantlet regeneration rates in palms are rather low and erratic (Arias, 1985; Rabéchault and Martin, 1976; Wooi et al., 1982b). A few large corporations

produce oil palms via tissue culture on a commercial scale, and although data published by these companies lack specific information about their laboratory techniques, it is this author's guess that their success does not derive from having found "magic" media that produce plantlet regeneration in high proportions, but rather that their operations are large enough to absorb the costs of the rather inefficient cloning process. A summary of the in vitro research carried out by various scientists on the coconut, oil, date, and pejibaye palms follows below.

Coconut (Cocos nucifera) tissue culture

Most of the research on coconut tissue culture has been conducted on zygotic embryos. The in vitro propagation of embryos does not, of course, eliminate any of the problems that can theoretically be avoided through asexual propagation, which are due to the segregation of parental genotypes. For the purposes of this thesis, therefore, embryo culture will not be reviewed in any detail. It should be noted, however, that embryo culture is important as a method to overcome problems such as premature fruit abortion, reduced fertility resulting from interspecific hybridization, or to shorten extended germination periods due to seed quiescence or dormancy.

Anther culture has been carried out in coconut (Tuyen and Guzman, 1983, in Paranjothy, 1986; Monfort, 1985), and the development of embryoids has been observed. Monfort surveyed more than 200,000 anthers. He observed androgenesis after 20 weeks in culture on Miller's, 1963, medium containing kinetin or glutamine, provided activated charcoal and TIBA were added. However,

the formation of multicellular structures occurred at a very low rate (10 % at the most) and appeared to be heavily dependent on the individual anthers cultured.

Coconut stem, leaf, and inflorescence tissues have been evaluated for their ability to form calli in vitro. Eeuwens (1976) tested White, 1943, Heller, 1953, and Murashige and Skoog, 1962, tissue culture media against Y3, a medium developed especially for coconut culture, and found the latter to be superior in terms of explant growth and callus induction. He stated that "cell division in the upper part of cultured explants gave rise to a layer of white callus within a month". All media tested were supplemented with kinetin, gibberellic acid, and 2,4-dichlorophenoxyacetic acid. A major factor in the growth response of the coconut explants was "the source and concentration of inorganic nitrogen. A requirement for both a reduced form of nitrogen (ammonium) and high concentrations of nitrate could be clearly demonstrated." The Murashige and Skoog medium seemed to be inadequate solely because it contains deficient levels of certain additives, especially iodine.

In 1977, Eeuwens further modified the Y3 medium (1976) by changing the organic nutrient and hormone composition, and created a new medium, BMY3. The coconut inflorescence explants and the date palm stem and leaf petiole explants tested on this medium and its variations, showed adequate growth and some morphogenesis (root production), but callus production was extremely limited and could not be subcultured successfully.

Apavatjrut and Blake (1977) describe the in vitro culture of subapical stem tissue extracted from mature coconut palms. The basic nutrient medium used was developed from the Heller, 1953, and Murashige and Skoog, 1962, media, and it contained both kinetin and 2,4-D. As in the Y3 medium mentioned above, the

iodine concentration was high. The primary variables tested were the presence or absence of coconut milk, and various incubation temperatures and nitrogen sources. All three variables had a significant effect upon callus initiation. Best results were obtained in the presence of coconut milk, with nitrate and ammonia as nitrogen sources, when the incubation temperature was 31°C. The article does not mention what proportion of the explants formed callus on their surface, but it was noted that cell division was observed as early as one week after the cultures were first established. Groups of cells resembling embryos or proembryos were also observed.

According to the published records available to this author about pejibaye tissue culture, in no other instance except in the research carried out for this thesis, have pejibaye stem explants been cultured. The three articles described last are therefore of particular interest.

Oil Palm (Elaeis guineensis) tissue culture

Few articles on oil palm tissue culture present their materials and methods in any detail, and this is due to the fact that the bulk of the research carried out on oil palm culture is protected by the interests of private industry.

Callus production and embryogenesis have been obtained from root and leaf tissue of mature plants and young seedlings since the early 1970s, but it was not until 1976 that Rabéchault and Martin regenerated plantlets in vitro from foliar tissue, which was the first such report on the oil palm. Noiret et al. indicated in 1985 that no in vitro propagation of the shoot tip could be obtained, but the culture of tissue obtained from unopened leaves continued to be a success. The advantage of using foliar tissue rather than shoot tips as the

explant is that up to 2,000 such explants may be obtained from a single plant without having to sacrifice it in the process. Noiret et al. (1985) reported the production of callus along the veins of the foliar tissue in 20 to 80 % of the cases, with subsequent embryogenesis in 75 % of those calluses. According to the calculations of this author, embryogenesis was obtained in 15 to 60 % of all cultures established. Despite this wide range in results the propagation rate is apparently very high (500,000 embryoids per clone per year), because "embryoids can give rise to secondary embryoids" (Noiret et al., 1985). Although it is not clear what percentage of the somatic embryos actually yielded plants, about 90 % of the plantlets survived upon transfer to normal growing conditions.

Thomas and Rao (1985) likewise reported successful cloning of the African oil palm through leaf tissue culture. The explants were obtained from 6-month old seedlings and placed on an MS medium with 2,4-D ranging from 50 to 70 mg/l. Subculturable callus tissue was produced in 50 % of the cases within 8 weeks, and subsequent transfer to sequentially lower 2,4-D concentrations resulted in the formation of nodular callus masses which, when placed on basal MS medium with only 0.01 to 0.5 mg/l 2iP, yielded somatic embryos at a rate of about 50 per culture. Shoots were transferred to a half-strength MS medium supplemented with 1 mg/l of NAA and GA_3 each. Upon root production the plantlets were transferred to small paper cups with sterilized soil compost. No data on the final regeneration rate were presented.

A concise review of oil palm characteristics, uses, and tissue culture is presented by Krikorian and Kann (1986). These authors indicate that publications in this field seldom point to the fact that "the yields of plantlets produced are relatively low and the responsiveness uncontrolled and erratic at

best, especially when a wide range of genotypes is tested". The culture of zygotic embryos of the oil palm, which has received much attention as well, is also discussed in their article.

Date Palm (Phoenix dactylifera) tissue culture

Although early studies involving the culture of zygotic embryos and crushed apical meristem disks yielded some bud differentiation and revealed the potential importance of a high incubation temperature (35°C) and the presence of natural complex additions to the medium, it was not until the late 1970s that the methods for date palm tissue culture started to be perfected.

Tisserat (1979a) reports the successful culture of lateral buds, shoot tips, embryos, and stem and rachilla tissue cultured on a modified MS medium, on which the effect of three different auxins and activated charcoal were tested. Callus, root, and adventitious plantlet formation were observed, depending on the auxin concentration. Zygotic and somatic tissues alike responded positively to the media utilized, but lateral buds and shoot tips were especially prone to callus and subsequent plantlet production. The addition of activated charcoal to the medium was instrumental in bringing about callus production and morphogenesis. By 1981, Tisserat had established a reliable method for the rapid propagation of the date palm using a modified MS medium containing 3 mg/l 2iP, 100 mg/l 2,4-D, and 3 g/l activated charcoal. The precise reproduction rate, however, was not presented in his 1979 article. Tisserat (1981) also describes a method for successfully using cryopreservation of date palm calli, which is of potential importance for all palms cultivated in vitro.

Sharma et al. (1984) observed hundreds of asexual embryos in each culture that produced viable calli, which occurred in 25 % of the shoot tip cultures, and in 19 % of the cultures with axillary buds. These somatic embryos were obtained as early as 4 weeks after the cultures were first established on a modified MS medium containing activated charcoal, BA, 2,4-D, and other additives. This preliminary research had been updated by 1986, when free-living plants were derived from cell suspensions. The survival rate of plantlets in the soil was between 60 and 80%. The authors recommend cell suspensions for commercial propagation of the date palm.

Pejibaye palm (Bactris gasipaes) tissue culture

It was not until the pejibaye palm was "rediscovered" by scientists in the late 1970s and early 1980s, after being neglected for a long time as an important research subject, that the first studies of pejibaye tissue culture began to emerge. Huete et al. (1981) and Arias and Huete (1983) were the first to carry out in vitro studies on this palm. In 1981 Huete et al. reported rather unsuccessful attempts at growing calli from leaf and root sections, but in 1983 Arias and Huete used seedling shoot tips as explants and reported a 10 to 33 % plantlet regeneration rate from embryoid structures after 8 to 12 months in culture, with an average of 3 to 5 plantlets obtained per embryogenic callus (Arias and Huete, 1983). They utilized a modified MS medium containing a combination of either NAA and KN, or 2,4-D and BA. Plantlet regeneration rates were comparable in both cases.

In 1985 Arias presented a survey of all pejibaye explants tested in the tissue culture laboratory of the University of Costa Rica. Female and male

inflorescences, rachises, rachillae, flower buds, and immature leaf explants gave no response when grown on a modified MS medium, prepared as described in Arias and Huete (1983), and Chaverri and Arias (1984, unpublished, cited in Arias, 1985). All cultures were grown in the dark. Root tip explants grown under the same conditions exhibited some root growth, while callus production was observed on immature and mature zygotic embryos and shoot apices. Plantlet regeneration upon transfer to light resulted only from the cultures of mature zygotic embryos and shoot tips. The use of casein hydrolysate as an oxidation suppressant and the use of alternative nitrate sources are reported. Some experiments with important levels of callus production (the rates were not specified) produced organogenic calli at more than 30 %, and calli with proembryonic structures at a rate of at least 8 %.

Villalobos and Arias (1986), cited in Pinedo Panduro (1987), reported the formation of two types of callus, one friable and the other compact, obtained from mature embryo explants cultured on 0.04 mg/l picloram. Compared to somatic tissue, for which high 2,4-D concentrations are required for the formation of callus, mature embryos required only 2.5 mg/l 2,4-D. NAA at 3 mg/l resulted in the formation of calli and roots, on which vegetative buds developed subsequently. All other treatments inhibited shoot proliferation.

Pasberg-Gauhl (1986) was unable to obtain either callus production or root development in shoot tips obtained from basal offshoots and cultured on NAA (0 to 100 mg/l) and BA (0 to 100 mg/l). These shoot tips showed very limited growth or development after 4 months. The author indicated that shoot tips obtained from seedlings, on the other hand, were better capable of responding in terms of growth and leaf development.

Salazar (1986) grew pejibaye shoot tips obtained from basal offshoots on modified MS supplemented with 2,4-D (0.1 to 100 mg/l) and BA (5 mg/l). The primary purpose of his research was to test the effect of light and activated charcoal on his cultures. He obtained complete plantlets after 4 to 5 months of incubation only in cultures containing activated charcoal. He observed a proliferation of axillary buds at 3 mg/l 2,4-D without activated charcoal, and at levels above 3 mg/l 2,4-D in the presence of charcoal (2,4-D levels above 3 mg/l in the absence of activated charcoal were inhibitory to axillary bud proliferation). Embryoids were obtained at 30 and 100 mg/l 2,4-D in the presence of activated charcoal in the dark. Salazar also tested young leaf and flower bud explants, but collected no data due to a substantial loss of cultures. He indicated, however, that young leaf tissue produced callus in the dark at 30 to 50 mg/l 2,4-D. His is the first published report on callus production from pejibaye leaf tissue.

Pinedo Panduro (1987) worked with pejibaye shoot tips and immature male flowers taken from mature and juvenile plants. Cultures were established on a modified MS medium with 10 mg/l NAA, or on a modified MS medium with 3 mg/l NAA and 0 to 21 mg/l BA. The former medium yielded organogenesis in only a few shoot tips which developed into complete plantlets after 7 months in culture and were successfully transferred to greenhouse conditions. In the latter case, an increase in the diameter of the shoot tips was observed in all cultures, followed by the formation of protuberances, which, after 9 months, became vegetative shoots in 54 % of the cases. The highest organogenesis rates were observed on treatment containing 3 mg/l NAA and 14 and 21 mg/l BA for adult plants, and 3 mg/l NAA and 7 mg/l BA for juvenile plants. Pinedo Panduro indicated that shoot

developmen may have resulted from pre-existing buds or from adventitious buds that developed de novo. No intermediary callus phase was observed in any culture, which allowed the author to conclude that no somaclonal variation was to be expected. He also attempted without success to induce organogenesis in floral buds using GA, BA, IBA, NAA, and 2,4-D.

Recently, Valverde et al. (1987) used a MS medium with picloram at 0.06 mg/l, BA at 5 mg/l, and coconut milk to induce calli on pejibaye shoot tips (picloram is the common name for the systemic herbicide 4-Amino-3,5,6-trichloropicolinic acid, classified as a restricted use pesticide in the United States) (Sine, 1987). The picloram concentration was reduced to 0.03 mg/l after 3 months. Of the original 100 cultures, 62 survived, and 42 of those cultures showed "very good callus formation". Severe browning developed on the cultures once they were transferred to light, but this browning was superseded by new, more vigorously growing calli which formed on the surface of the old, darkened tissue upon transfer of the cultures to a medium devoid of hormones. These cultures continued to be subcultured on one-month intervals, and it was possible to subdivide some of them. Of the 42 cultures with optimal callus growth, 32 demonstrated organogenic activity only, while the other 10 exhibited both organogenic development and the production of somatic embryos. The latter were easily separated from the cultures and grown individually. It was possible to transfer the plantlets developing from those embryos to greenhouse growing conditions, if they were first aseptically grown on peat-moss moistened with half-strength MS salts. The final survival rate of plantlets transferred to greenhouse conditions was about 80 %. The most productive callus culture yielded 8 somatic embryos, 24 organogenetic plantlets, and 10 primordia. The least productive, on

the other hand, produced 2, 4, and 3 respectively. According to the calculations of this author, at least 13 months elapsed between the establishment of the original cultures and the transfer of the first plantlets to the sterile peat-moss environment. This study by Valverde et al. is the most recently published on pejibaye tissue culture, and the one that provides the most convincing evidence so far of the occurrence of somatic embryogenesis in this species.

Statement of Problem

Monocotyledons are generally difficult to propagate by in vitro techniques, palms being perhaps the most recalcitrant among the monocotyledons (Ammar and Benbadis, 1977; Krikorian and Kann, 1986; Hunault, 1979; Jones, 1974; and Reuveni and Lilien-Kipnis, 1974). However, palms are among the most important tropical species due to their multiplicity of uses and their role as oil crops. For this reason it is important to persist in the tissue culture research. A better understanding of the problems of palm propagation and higher success rates in reproduction are bound to result from perseverance.

The objectives of the research presented here are: a) to compare shoot-tip, stem, and leaf-sheath explants in terms of their responsiveness in vitro, b) to study general callusing behavior in the pejibaye palm, c) to evaluate the effect of certain organic medium additions in terms of their browning-reduction or growth-promoting characteristics, and d) to formulate recommendations for media to use in the tissue culture of the tissues that have cloning potential. It is hoped that the material presented in this thesis will contribute useful information to these ends.

Explanation of Thesis Format

This thesis has been written in the so-called alternate format, which means that the text has been subdivided into discrete parts, each encompassing a separate topic, instead of being presented as a continuous manuscript in which all subjects are covered together. To the reader, this format has the advantage of making any specific topic easily accessible, and discussing all matters related to that topic within contiguous pages.

The preceding literature review, and the Summary and Discussion section at the end of the thesis, have been included in order to provide a general context for each of the subdivisions. The reader may be interested in a specific topic, but she or he may also want to know how this topic relates to other areas in the same general field of study. The General Bibliography (p. 150) includes not only sources consulted in the writing of this document, but also other references related to the topic, which the reader might find useful. The annotated bibliography compiled by Clement and Arias de Guerrero (1983) represents a complete list of publications about the pejibaye palm, or publications in which the pejibaye palm is mentioned, up to 1983.

Two abstracts are included in this thesis: one in English and a translation in Spanish. Since most of the research on the pejibaye palm has been carried out in Spanish-speaking countries, this author deems it appropriate to include an abstract in the Spanish language for the benefit of researchers who may not speak or read English.

Abbreviations

The following abbreviations were used throughout the text:

BA = Benzyladenine

MS = Murashige and Skoog

NAA = 1-Naphthaleneacetic acid

2,4-D = 2,4-Dichlorophenoxyacetic acid

ENGLISH ABSTRACT

Pejibaye (Bactris gasipaes H.B.K.) stem, leaf-sheath, and shoot-tip explants, taken from spiny and smooth-stemmed seedlings, were cultured on a variety of media containing 2,4-D and BA in various combinations, with the purpose of studying callus formation and possible embryogenesis. Activated charcoal and coconut water were included in some studies.

Leaf-sheath sections had their highest callus initiation rates on a modified MS medium containing 25 mg/l of 2,4-D and 5 mg/l of BA. These calli, however, did not grow well and failed to produce any organized structures. Smooth-stemmed palms gave significantly higher callus initiation rates.

Subapical stem explants produced far better calli than leaf-sheath explants, especially when grown on media containing high BA:2,4-D ratios. Globular callus structures, sometimes accompanied by root initiation, were observed on media containing between 4 and 12 mg/l BA and 1 and 7 mg/l 2,4-D, with or without 100 ml/l coconut water. In these cultures, significantly higher callusing rates were observed in spiny-stemmed palms. Both leaf-sheath and subapical stem sections produced calli only on the cut surface facing away from the medium.

Shoot tips gave the best callusing response. Levels of 25-50 mg/l 2,4-D and 5 mg/l BA, with or without 100 ml/l coconut water, proved most satisfactory. Direct organogenesis (no callusing) was observed on the same media combinations in the presence of 2.5 g/l activated charcoal. Callusing rates were significantly higher in explants of 4-month-old seedlings than in explants of 1½-year-old

seedlings. No callusing differences between smooth-stemmed and spiny-stemmed palms were observed in shoot-tip cultures.

Browning was a problem with leaf-sheath and stem tissue cultures, but not with shoot-tip cultures. Browning of stem tissue was never eliminated, although it was partially controlled by frequent subculturing, using Gelrite at 2 g/l as the solidifying agent.

SPANISH ABSTRACT: COMPENDIO

Apices caulinares y secciones de la base foliar y de tallo extraídos de plantas de pejibaye (Bactris gasipaes H.B.K.), la mayoría de ellas de 1½ años de edad, fueron cultivados sobre el medio de Murashige y Skoog (1962) modificado con 2,4-D y BA en varias combinaciones, con el propósito de estudiar la formación de callo y posible embriogénesis somática. En algunos estudios se incluyeron carbón activado y agua de coco. Se compararon plantas de tallo liso con plantas de tallo espinoso.

La mejor tasa de iniciación de callos en explantes de base foliar se dio sobre el medio MS modificado con 25 mg/l de 2,4-D y 5 mg/l de BA. Plantas de tallo liso produjeron significativamente más callos que plantas de tallo espinoso. Sin embargo el crecimiento de estos callos no fue satisfactorio y no produjo estructuras organizadas.

Los explantes de tallo produjeron callos de crecimiento activo sobre medios con proporciones altas de citocinina:auxina. Callosidades globulares, acompañadas a veces por inicios radicales, fueron observadas en el medio MS modificado con 4-12 mg/l de BA y 1-7 mg/l de 2,4-D, con o sin 100 ml/l de agua de coco. En este estudio palmas de tallo espinoso produjeron callos en porcentajes significativamente mayores. Tanto los explantes de base foliar como los de tallo produjeron callos únicamente sobre la superficie de corte superior.

La mejor producción de callos se obtuvo en ápices caulinares. EL medio MS modificado con 25 a 50 mg/l de 2,4-D, 5 mg/l de BA, con o sin 100 ml/l de agua de coco, produjo el mejor crecimiento de callos. Se observaron varios

tipos de organogénesis directa, sin producción intermedia de callo, sobre los medios que contenían 2.5 g/l de carbón activado.

La oxidación de explantes fue problemática en secciones de la base foliar y de tallo, pero no en los ápices caulinares. El problema de la oxidación no se pudo eliminar, aunque sí se logró controlar en parte por medio del trasplante frecuente de los cultivos, usando 2 g/l de Gelrite como gelificante.

PART I. PEJIBAYE EXPLANT BROWNING

ABSTRACT

Leaf-sheath sections of 1-year-old pejibaye seedlings were placed on MS media containing 10 mg/l 2,4-D and 5 mg/l BA. The treatments consisted of different concentrations of the solidifying agents Difco-Bacto agar (used at 2, 4, 6, and 8 g/l) and Gelrite (used at 1, 2, 3, and 4 g/l). Browning was worst on 8 g/l Difco-Bacto agar and least pronounced on 1 g/l Gelrite. Due to the inadequate firmness of Gelrite at 1 g/l, the use of 2 g/l Gelrite is recommended, despite the slightly higher browning rate.

The incidence of shoot-tip and leaf-sheath browning was also tested on media containing either activated charcoal, casein hydrolysate, or citric acid, and in explants which were subcultured weekly or soaked in an aqueous solution of citric and ascorbic acid prior to being placed on the medium. The basal medium in all cases was an MS formulation with 10 mg/l 2,4-D, 5 mg/l BA, and 100 ml/l coconut water. No significant differences were found among treatments or between shoot-tip and leaf-sheath explants.

INTRODUCTION

Browning in palm tissue culture is a common problem (Apavatjrut and Blake, 1977; Tisserat, 1981; Arias and Huete, 1983; Sharma et al., 1986; Valverde et al., 1987). This phenomenon may be explained by the presence of copper ions in agar, which are utilized by the copper-containing enzyme complex phenolase. This enzyme complex is responsible for the oxidation of endogenous phenols, a chemical process which in most cases is the cause for browning in wounded tissue (Debergh, 1983, and Rhodes et al., 1978). The effects of browning may be quite severe and lead to the death of the tissue involved (Sharma et al., 1986). In other instances, even when the explant shows complete browning, the tissue survives and even overcomes the problem by producing fresh cells which continue to respond to the in vitro conditions (Valverde et al., 1987). Browning may also occur to an intermediate degree and can in some cases be prevented from spreading by frequent subculturing of the explant or the addition of certain organic compounds to the medium, such as activated charcoal (Tisserat, 1984).

Agar is a mixture of polysaccharides derived from extracts of several species of red algae (Kyte, 1983). It frequently contains traces of elements that constitute impurities (Debergh, 1983; Kohlenbach and Wernicke, 1978; Kyte, 1983; Singha, 1984; Stoltz, 1971). Growth of explants is inhibited as the agar concentration increases, which may be due to the fact that water and nutrients in the medium are more firmly bound in the agar at higher concentrations, and therefore are less available to the tissue (Kohlenbach and Wernicke, 1978; Romberger and Tabor, 1971; Stoltz, 1971; Von Arnold and Ericksson, 1984). Agar may also interact with cytokinin by making it less available to the plant tissue

at higher concentrations of agar (Debergh, 1983). Several agar substitutes have been developed which do not contain as many undefined substances as Difco-Bacto agar. One such compound is Gelrite, a solidifying agent derived from bacteria (Kyte, 1983). At least three authors (Debergh, 1983; Singha, 1984; Pinedo Panduro, 1987) have indicated the desirability of using such agar substitutes whenever agar seems to affect cultures adversely.

Several compounds have been found to reduce browning in palm tissue culture. They include activated charcoal (Monfort, 1985; Salazar, 1986; Tisserat, 1984), and casein hydrolysate (Arias, 1985). In other cases, frequent subculturing (Tisserat, 1984) or pre-soaking of the explant in citric and ascorbic acid (Sharma et al., 1986) have been used. The purpose of this study was to examine the role played by the solidifying agent and various anti-oxidation treatments in the reduction or enhancement of pejibaye explant browning.

MATERIALS AND METHODS

Experiment 1:

The Effect of Solidifying Agent Brand and Concentration

Two different gelling or solidifying agents were tested in this experiment: Difco-Bacto agar and Gelrite agar substitute. Media containing four different concentrations of each gelling agent were prepared, yielding a total of eight different treatments. The basal medium in all eight treatments was the 1962 Murashige and Skoog formula, to which 10 mg/l 2,4-D and 5 mg/l BA were added. The four concentrations of Difco-Bacto agar were 2, 4, 6, and 8 g/l. Gelrite was added in amounts of 1, 2, 3, and 4 g/l. After the pH had been adjusted to 5.8, the media preparations were autoclaved for 15 minutes at 1.1 kg/cm^2 and 121°C, and then dispensed into disposable 6-cm petri dishes in 15-ml aliquots.

A total of five 1-year-old seedlings were used in this randomized complete block design. One block consisted of eight explants obtained from a single seedling, each of which was assigned at random to one of the eight treatments. Therefore, each seedling represented one block, and each of the five blocks contained all eight treatments.

The seedlings were imported from Costa Rica when they had just emerged from the seeds, and were grown in individual pots in a greenhouse at 27°C. No artificial light was used. The seedlings were watered every other day and fertilized every other week with a soluble greenhouse fertilizer containing 200 ppm N. Iron chelate was added to the fertilizer if chlorosis symptoms appeared.

Explants were obtained by cutting off the roots, foliage, and part of the stem of the palm seedlings, until 9-cm stem segments were left which included the broad stem base and leaf-sheath tissue. These segments were rinsed several times to remove the soil. The outermost tissue layer of each stem segment, i.e the base of a leaf sheath, was peeled off, and the stem segments were subsequently surface sterilized by immersing them in 70 % ethyl alcohol for one minute. This step was followed by a transfer of the stem segments into a 30 % (v/v) commercial chlorine bleach solution containing 2 drops of the surfactant "Tween 80", in which the segments were shaken continuously for 15 minutes. This step was followed by 3-4 rinses in sterile deionized water under aseptic conditions. Two more tissue layers or leaf sheaths were then removed from the segments, which left stem cylinders formed by the inner two or three layers of leaf-sheath tissue enclosing the leaf primordia, with the broad stem base still attached at the bottom or proximal end. At this point the broad stem bases were cut off, leaving cylinders made up of leaf-sheath tissue only. The primary reason for leaving the stem bases attached thus far was to prevent contamination of the inner layers of the leaf-sheath tissue during preparation of the segments.

Eight slices were cut from the proximal end of each leaf-sheath cylinder, as shown in PART II of this thesis. Each such slice was then randomly placed on one of the eight media. The slices had a diameter of about 1 cm and were about 0.2 cm thick. The cultures were placed in a dark growth chamber at 24±1°C, from which they were removed for observation once a week. Browning was evaluated on a subjective scale in which the score of 0 indicated no browning; 1, light browning on the bottom and along the edges of the explant; and 2 (Fig. I-1a), dark browning on the bottom and along the edges, with some browning on the

upper cut surface. Subculturing was carried out once after 5 weeks, and final data were taken at the end of 9 weeks. Histological sections were prepared by fixing the plant tissue, dehydrating it and embedding it in paraffin, after Johansen (1940). Tissue slices were cut on a microtome at settings of 10 and 15 µm, mounted on microscope slides and stained as indicated, using safranin and chlorazol black E as stain and counterstain respectively (Johansen, 1940).

Experiment 2:

Addition of Anti-Oxidants and Other Treatments

An experiment was set up in which browning on shoot-tip explants was compared to browning on leaf-sheath sections on six treatments, all designed to reduce browning of pejibaye explants in vitro. Shoot tips and leaf-sheath sections were taken from both spiny and smooth-stemmed palm seedlings in a split-plot experimental design. Five replications were used per explant per treatment. The 1962 Murashige and Skoog tissue culture medium was used in all cases, with the addition of 10 mg/l 2,4-D, 5 mg/l BA, and 100 ml/l coconut water. On the control treatment, the explants were left on the culture medium without being handled any further. In the second treatment, explants were soaked in a sterile aqueous solution of citric and ascorbic acid (150 mg/l each) for 5 minutes prior to being plated out. The third treatment consisted of a weekly subculture of the explants to fresh medium. None of the other treatments were subcultured during the 8 weeks of observation. Citric acid at 150 mg/l was added to the medium in the fourth treatment, 400 mg/l casein hydrolysate to the medium in the

fifth treatment, and 2.5 g/l activated charcoal to the medium in the sixth treatment.

The source of the plant material was identical to that described under Experiment 1, as were the greenhouse conditions and the surface sterilization procedure. To obtain the shoot tips, stem segments were held by hand at their distal end, where the foliage was originally attached, and thin slices were cut from the broad stem base. Contamination was avoided by holding the distal end outside the petri dish, while resting the proximal end inside the petri dish in which the dissection was carried out. After a few slices had been removed from the broad stem base, several concentric "rings" became visible in the broad stem tissue. This indicated that the cuts had reached an area very close to the shoot tip. At this point a circle was cut into the stem tissue following the contour of the innermost "ring" visible in the center of the cut stem surface. By squeezing the base of the stem section gently between two fingers, the shoot tip, with a thin layer (1-2 mm) of stem tissue still attached at its base, was ejected from the stem section without suffering any visible damage.

Leaf-sheath sections were obtained by removing the rest of the stem base tissue and the outermost leaf sheath, which had been squeezed between the fingers during the extraction of the shoot tip and was therefore presumed to be contaminated. Subsequently, slices were cut from the proximal end of the remaining leaf-sheath cylinder as described in Experiment 1.

One shoot tip and one leaf-sheath section were obtained from each of four seedlings and were assigned at random to one of the six treatments. Shoot-tip cultures represented one half of the split-plot design, leaf-sheath sections the other. A similar browning scale to that of Experiment 1 was applied, with the

addition of a third browning score, 3, for cases in which browning covered the entire explant. The incubation conditions and observation schedule were the same as for Experiment 1.

RESULTS

Experiment 1:

The Effect of Solidifying Agent Brand and Concentration

Mild browning became visible for the first time in some cultures during the third week and progressed slowly during the fourth week. It intensified and became more widespread just before and after subculture in the fifth week, and continued to do so until it stabilized around the seventh week. Differences among treatments were significant, as were differences among seedlings, i.e., blocks (Table I-1). The gelling agent brands used affected browning significantly (Table I-1), with the mean browning score for Gelrite being about half of that of Difco-Bacto agar (Table I-2). Browning increased steadily with increasing concentrations of Difco-Bacto agar. With Gelrite, however, the relationship between concentration and browning was not direct, because browning was higher on 3 g/l than on 4 g/l (Table I-2). The lowest browning mean occurred on 1 g/l Gelrite, wheras the highest browning mean was observed on 8 g/l Difco-Bacto agar (Table I-2). Significant browning differences were manifested among the 5 seedlings, i.e. among the 5 blocks of this experiment (Table I-1).

Callusing was limited to the upper cut surface of all explants, and by the eighth week it had not progressed noticeably. There was never more than 1 mm of callus "icing" on the explants. In seven cultures (17.5 % of the total) the calli were no longer evenly distributed over the cut surface, but were interrupted by a tissue formation which resembled the texture of a sponge (Fig. I-1). Two of these "spongy" cultures developed on 4, 6, and 8 g/l Difco-Bacto agar

each, and one on 3 g/l Gelrite. A histological section of "spongy" tissue revealed empty spaces with ruptured cells in between vascular bundles (Fig. I-2).

The growth pattern of most leaf-sheath explants was characterized by a slow initial enlargement during the first four days in culture, followed by a roughly 5-fold size increase between the end of the first week and the end of the fourth week, after which the explants ceased to enlarge any further.

Experiment 2:
Addition of Anti-Oxidants and Other Treatments

Browning was present on all six treatments, and the analysis of variance indicated that no significant differences existed among treatments or between shoot tips and leaf-sheath sections.

Superficial callusing was observed in most leaf-sheath sections at the end of the second week. The shoot tips started to show signs of callus initiation after the third week in culture. The enlargement pattern of the leaf-sheath sections was similar to that in Experiment 1, with a rather dramatic increase in the size of the explants, both in diameter and height, taking place during the second and third weeks of incubation, followed by an apparent reduction of the growth rate. Shoot tips did not grow fast at any point, although their bases increased in diameter during the third week in culture.

DISCUSSION AND CONCLUSIONS

The results of Experiment 1 indicate that Difco-Bacto agar affects pejibaye leaf-sheath cultures adversely, especially at a concentration of 8 g/l (Table I-2), which is a very common formulation used in tissue culture. Although Gelrite at 1 g/l produced the least browning, it is recommended that 2 g/l Gelrite be used, unless extremely small meristem sections are used as explants. Gelrite at a concentration of 1 g/l was not firm enough to support enlarged pejibaye shoot tips or stem sections, and they tended to sink into the medium. This may limit the amount of oxygen that reaches such cultures, which in turn would be detrimental to their development. Although 2 g/l Difco-Bacto agar was associated with a relatively low browning mean (Table I-2), its use is discouraged, too, because it did not solidify, and the explants died during the observation period.

The formation of sponge-like tissue, which was observed mostly on media solidified with Difco-Bacto agar, may be due to a certain degree of desiccation of the upper cut surface of the explant at a time when cultures go through their rapid growth phase. Such desiccation may cause fast-growing cells to rupture, as shown in Fig. I-2. This hypothesis is supported by Debergh's (1983) observation that high concentrations of agar tend to bind water more firmly and therefore make it less available to the explant. Usually callus initiation recedes as "sponginess" progresses, as observed by this author in other tissue culture experiments involving leaf-sheath sections.

Although several workers have used or suggested activated charcoal, casein hydrolysate, citric and ascorbic acids, and frequent subculturing as ways to prevent or limit tissue browning in palms, Experiment 2 indicates that none of

these measures consistently prevent browning in pejibaye tissue culture. As a result, no recommendations can be made if the sole purpose of adding those substances to the medium is to prevent browning. The exception is frequent subculturing, which has aided this author in controlling medium discoloration whenever browning was not confined to the explant, even though the browning of explants *per se* was not thereby reduced.

REFERENCES CITED

Apavatjrut, Pimchai, and J. Blake. 1977. Tissue culture of stem explants of coconut (Cocos nucifera L.). Oléagineux 32(6):267-270.

Arias, Oscar. 1985. Propagación vegetativa por cultivos de tejidos del pejibaye (Bactris gasipaes H.B.K.). ASBANA 24(9):24-27.

Arias M., Oscar, and Fernando Huete V. 1983. Propagación vegetativa in vitro de pejibaye (Bactris gasipaes H.B.K.). Turrialba 33(2):103-108.

Debergh, P. C. 1983. Effects of agar brand and concentration on the tissue culture medium. Physiol. Plant. 59:270-276.

Johansen, Donald Alexander. 1940. Plant microtechnique. McGraw-Hill, New York. 523 pp.

Kohlenbach, H. W., and W. Wernicke. 1978. Investigations on the inhibitory effect of agar and the function of active carbon in anther culture. Z. Pflanzenphysiol. 86:463-472.

Kyte, L. 1983. Plants from test tubes. Timber Press, Portland, Oregon. 132 pp.

Monfort, S. 1985. Androgenesis of coconut: Embryos from anther culture. Z. Pflanzenzüchtg. 94:251-254.

Murashige, Toshio and Folke Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15(3):473-497.

Pinedo Panduro, Mario Herman. 1987. Organogénesis directa en ápices caulinares de pejibaye (Bactris gasipaes H.B.K.). Master's Thesis. Centro Agronómico Tropical de Investigación y Enseñanza, Turrialba, Costa Rica. 110 pp.

Romberger, J. A., and C. A. Tabor. 1971. The Picea abies shoot apical meristem in culture. I. Agar and autoclaving effects. Am. J. Bot. 58(2):131-140.

Salazar, Sebastián S. 1986. The effect of 2,4-D, light, and activated charcoal on offshoot-tip culture of the pejibaye palm (Bactris gasipaes H.B.K.). Master's Thesis. University of Birmingham, Birmingham, England. 70 pp.

Sharma, D. R., Sunita Deepak, and J. B. Chowdhury. 1986. Regeneration of plantlets from somatic tissues of the date palm Phoenix dactylifera Linn. Ind. J. Exp. Biol. 24:763-766.

Singha, S. 1984. Influence of two commercial agars on in vitro shoot proliferation of 'Almey' crabapple and 'Seckel' pear. HortScience 19:227-228.

Stoltz, Leonard P. 1971. Agar restriction of the growth of excised mature iris embryos. J. Am. Soc. Hort. Sci. 96(5):681-684.

Tisserat, Brent. 1981. Date palm tissue culture. U.S. Department of Agriculture. Agricultural Research Service. Advances in Agricultural Technology. Western Series. No. 17. (U.S. Department of Agriculture, Oakland, Calif.). 50 pp.

Tisserat, Brent. 1984. Clonal propagation: Palms. Pages 74-81 in Vasil, Indra K., ed. Cell culture and somatic cell genetics of plants. Vol.1. Academic Press, Inc., New York.

Valverde, Roberto, Oscar Arias, and Trevor A. Thorpe. 1987. Picloram-induced somatic embryogenesis in pejibaye palm (Bactris gasipaes H.B.K.). Plant Cell, Tissue and Organ Culture 10:149-156.

Von Arnold, S., and T. Ericksson. 1984. Effect of agar concentration on growth and anatomy of adventitious shoots of Picea abies (L.) Karst. P. C. T. O. Culture 3:257-264.

Table I-1. Analysis of variance for browning of pejibaye leaf-sheath explants on 10 mg/l 2,4-D and 5 mg/l BA. ("SDLNG" stands for seedling and refers to the differences among seedlings, i.e., blocks. "TRT" stands for treatment and refers to the use of Difco-Bacto agar and Gelrite at 4 different concentrations each. "CONC" refers to the different Difco-Bacto agar and Gelrite concentrations used. "BRAND" refers to the gelling agent used, i.e., Difco-Bacto agar versus Gelrite)

Source	df	Sums of Squares	f Value
SDLNG (=Blocks)	4	9.15	5.90***
TRT	7	10.77	3.97**
CONC	3	4.67	4.02*
BRAND	1	4.22	10.90**
BRAND*CONC	3	1.87	1.61$^{n.s.}$
ERROR	28	10.85	
CORRECTED TOTAL	39	30.77	

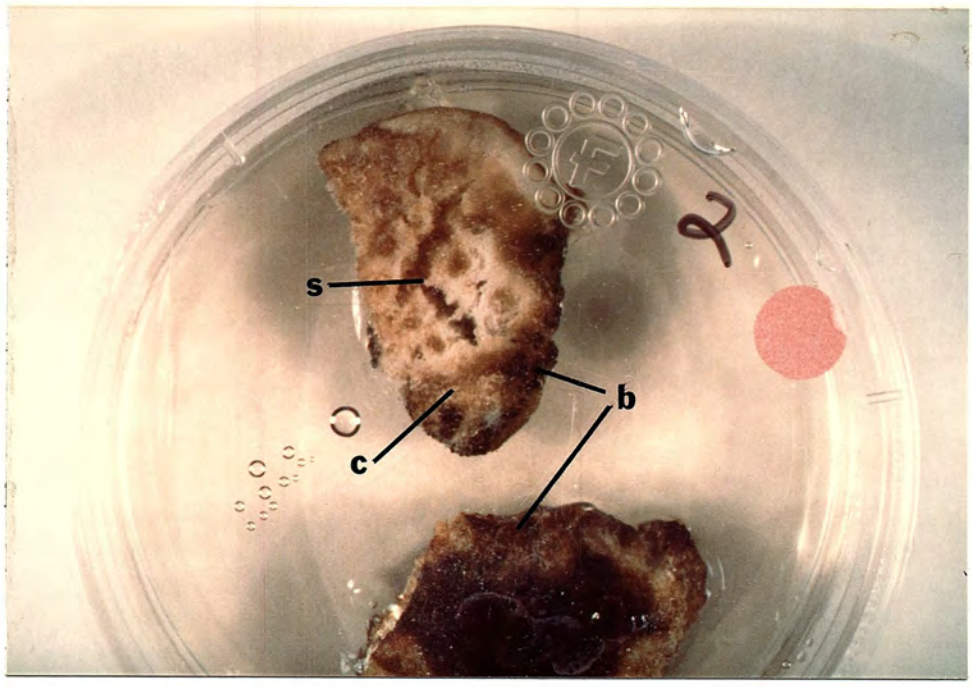
*Significant at the 5 % level.
**Significant at the 1 % level.
***Significant at the 0.1% level.
$^{n.s.}$Non-significant.

Table I-2. Browning means on pejibaye leaf-sheath explants for the eight treatments containing either Difco-Bacto agar or Gelrite as solidifying agents. (The means were derived from scores which were assigned as follows: 0 = no browning, 1 = light browning, mostly on the bottom surface and along the edge of the explant, and 2 = dark browning on the bottom and along the edges, with some browning on the upper surface of the explant.)

GELLING AGENT	**CONCENTRATION** (g/l)	**BROWNING MEAN**
Difco-Bacto agar	2.0	0.80
"	4.0	1.60
"	6.0	1.20
"	8.0	2.00

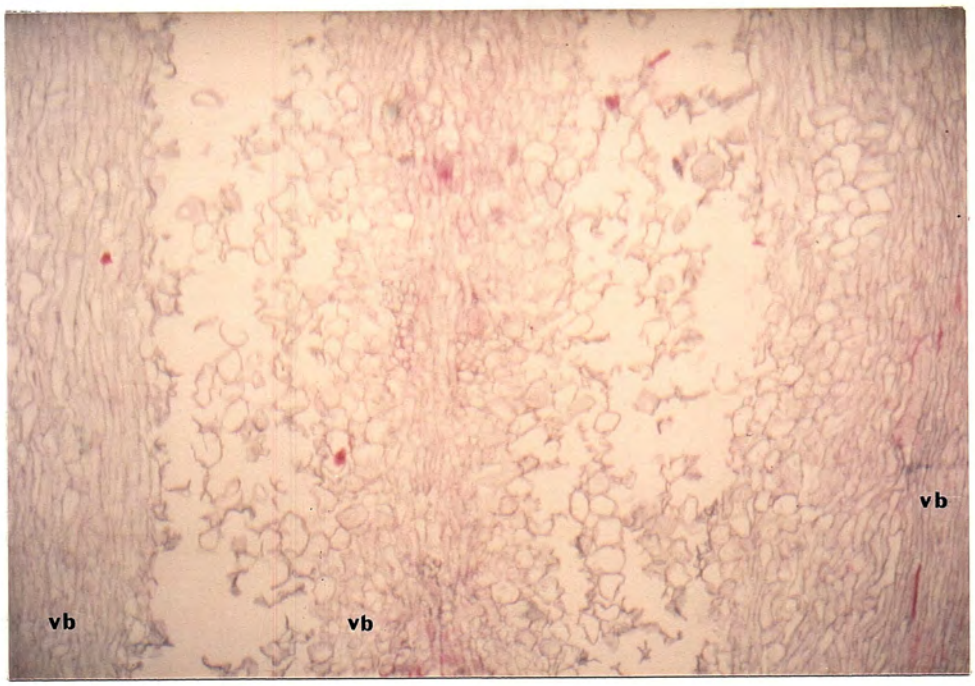
	Mean	1.40
Gelrite TM	1.0	0.20
"	2.0	0.80
"	3.0	1.20
"	4.0	0.80

	Mean	0.75



b = browning
c = callus
s = "spongy" tissue

Figure I-1. Formation of sponge-like tissue on pejibaye leaf-sheath explants. The browning level in this photograph has a score of 2 (dark browning on bottom, along edges, and some on upper cut surface). Some superficial callusing is visible as a cream-colored "haze"



vb = vascular bundles

Figure I-2. Longitudinal section of "spongy" tissue on a pejibaye leaf-sheath explant showing ruptured cells and empty spaces in between vascular bundles. (Magnification: 20.5x)

PART II. TISSUE CULTURE OF PEJIBAYE LEAF-SHEATH EXPLANTS

ABSTRACT

Callus initiation on leaf-sheath tissue was tested on MS (1962) media containing 0, 25, 50, 75, and 100 mg/l 2,4-D and 0 or 5 mg/l BA. Callus initiation and browning were highest on 25 mg/l 2,4-D and 5 mg/l BA. Discoloration of the explants occurred on the control medium (neither 2,4-D nor BA) and on media containing high 2,4-D levels (50-100 mg/l). Discoloration and browning were inversely related. Significant differences in callus initiation were found between spiny and smooth-stemmed palms. Callus production failed to develop beyond initiation and did not produce organized structures. Histological sections revealed that calli consisted of very large, unevenly-shaped cells with little or no meristematic activity. Explant orientation and exposure to light did not affect callus initiation.

INTRODUCTION

The leaf axis in palms consists of "three distinct parts, the rachis bearing the leaflets, a long or short petiole, and the basal leaf-sheath...[which, at] its insertion[,] always completely encircles the stem, but above[,] the sheath may be either open or closed....When closed[,] the leaf sheath is tubular and clearly delimited from the petiole" (Tomlinson, 1961). Members of the genus Bactris have closed or tubular leaf sheaths (Tomlinson, 1961). No reference to the use of leaf-sheath explants in palm tissue culture was found by this author. Due to the fact that leaf sheaths and rachises are part of the same organ, the leaf, and because of the functional similarities between the rachis (it supports the leaflets) and the rachilla (it supports the inflorescences and later the fruits), literature on rachis and rachilla tissue is reviewed here. Rachis and rachilla tissue cultures in palms have produced a variety of responses, including root growth (Eeuwens, 1978, and Tisserat, 1979), embryogenesis (Branton and Blake, 1983), and whole plantlet regeneration (Thomas and Rao, 1985). It is usually assumed that the best callusing may be obtained from rapidly dividing, meristematic cells, such as those found in shoot-tips (Hartmann and Kester, 1983, and Kyte, 1983), and not from highly differentiated cells, such as those found in stem or leaf tissue (Krikorian and Kann, 1986, and Tisserat, 1984). However, parenchyma cells have the ability to de-differentiate and form calli (Kyte, 1983), which may explain the success of rachis and rachilla cultures. Furthermore, a study conducted by Ahée et al. (1981) revealed that it may take over 2 years for mature tissue to obtain embryonic competence, suggesting that the lack of an immediate in vitro response in mature tissue does not necessarily imply its

inadequacy as an explant. One important advantage of the use of leaf-sheath, rachis or stem tissue over the use of shoot tips as explant sources is that several explants, either slices or blocks, may be obtained from a single plant if the former are used, whereas only one explant, namely the apical meristem and surrounding leaf primordia, can be obtained if the shoot tip is used. In species like the palms, where in vitro response rates are erratic and low (Ammar and Benbadis, 1977, and Krikorian and Kann, 1986), it is important to obtain as much explant material as possible from a given plant in order to maximize efficiency.

When preparing seedling stems for culture and cutting them into several slices, it is easy to be inconsistent in placing the stem slices with the same cut surface on the medium. One may place one slice with the proximal cut surface on the medium, and the next with the distal cut surface, i.e., "upside down". It was examined whether the effect of explant orientation on the medium is important in leaf-sheath tissue culture of the pejibaye palm. A study of the effect of light on callus formation on leaf-sheath explants was also carried out.

MATERIALS AND METHODS

Experiment 1:

Callus Induction on Leaf-Sheath Explants

Pejibaye leaf-sheath sections were placed on 6 treatments consisting of various combinations of 2,4-D and BA. The experiment was set up as a split-plot design, 7 replications of which tested the responses of smooth-stemmed pejibaye palm seedlings, and 7 more the responses of spiny-stemmed seedlings. There were six samples per treatment, so that 36 observations were collected per replication.

The basal medium used in all cases was a modified MS (1962) formulation containing 8 g/l Difco-Bacto agar. One treatment served as the control medium and contained no growth regulators. Each of the 5 remaining treatments contained 5 mg/l BA, with 0, 25, 50, 75, and 100 mg/l 2,4-D respectively. After the pH had been adjusted to 5.8, the media preparations were autoclaved for 15 minutes at 1.1 kg/cm^2 and 121°C, and dispensed into disposable 6-cm petri dishes in 15-ml aliquots.

A total of 84 1½-year-old seedlings were utilized in this experiment. Seedlings were imported from Costa Rica when they had just emerged from the seeds, and were grown in individual pots in a greenhouse at 27°C. No artificial light was used. The seedlings were watered every other day and fertilized every other week with a soluble greenhouse fertilizer containing 200 ppm N. Iron chelate was added to the fertilizer if chlorosis symptoms appeared.

Explants were obtained by cutting off the roots, foliage, and part of the stem of the palm seedlings, until 9-cm stem segments were left which included leaf-sheath tissue and the broad stem base of the seedling. These segments were rinsed several times to remove the soil. The outermost tissue layer (the base of a leaf sheath) of each stem segment was peeled off, and the stem segments were subsequently surface sterilized by immersing them in 70 % ethyl alcohol for one minute. This step was followed by a transfer of the stem segments into a 30 % (v/v) chlorine bleach solution containing 2 drops of the surfactant "Tween 80", in which they were shaken continuously for 15 minutes.

Upon completion of this step the segments were rinsed 3-4 times with sterile deionized water under aseptic conditions. Two more layers or leaf-sheath bases were then removed from the stem segments, which left cylinders formed by the inner two or three layers of leaf-sheath tissue enclosing the leaf primordia, with the broad stem base still attached at the bottom, or proximal end.

At this point the broad stem bases were cut off. The primary reason for leaving them attached thus far was to prevent contamination of the inner layers of the leaf-sheath tissue during preparation of the segments. The distal end of each remaining leaf-sheath cylinder, where the foliage had been attached, was then held by hand outside a petri dish while resting the proximal end inside. Explants were obtained by cutting 6 slices from the proximal end of each leaf-sheath cylinder (Fig. II-1a). Each such slice was then placed randomly on one of the 6 media. The slices had a diameter of about 1½ cm (Fig. II-1b) and were about 0.2 cm thick. The cultures were placed in a dark growth chamber at 24±1°C, from which they were removed for observation once a week. Subculturing was carried out every six weeks over a period of five months.

The three most important responses in this experiment-callusing, browning, and discoloration-were recorded as being either present or absent. Discoloration consisted of a complete lack of growth or change in the explant from its original form, accompanied by a fading of the original white-cream color to the point of acquiring an almost translucent quality. This experiment contained several missing values due to fungal or bacterial contamination, therefore Type III Sums of Squares were used in the analyses of variance.

Experiment 2:

Effect of Explant Orientation and Light on Callusing

Palm material was obtained, grown, and dissected as described in Experiment 1. The trial was set up as a factorial design consisting of two treatments at two levels each: orientation (up or down) and incubation condition (light or dark). A modified MS (1962) formulation containing 25 mg/l 2,4-D, 5 mg/l BA, and 2.0 g/l Gelrite was used as the culture medium in all cases. After the pH had been adjusted to 5.8, the medium was autoclaved for 15 minutes at 1.1 kg/cm^2 and 121°C, and dispensed into disposable 6-cm petri dishes in 15-ml aliquots.

Two leaf-sheath sections were obtained from each of ten 1½-year-old seedlings. One such section was placed with its proximal surface on the medium (right-side-up), the other with its distal surface (upside down). There were ten petri dishes containing right-side-up, and ten containing upside-down explants. Subsequently, half of the culture plates in each category were placed in the light under 1600 lux, 18 hours a day, the other half in a dark growth chamber.

Both growth chambers were kept at 24±1°C. The explants were subcultured once a month, and final observations were taken after 3 months. The presence or absence of calli and browning were scored.

Experiment 3: Histological Analysis

A 1½-year-old pejibaye seedling was dissected as described in Experiment 1, and three leaf-sheath slices were obtained from it under aseptic conditions. One of them was fixed immediately, then dehydrated and embedded in paraffin, after Johansen (1940). Tissue slices were cut on a microtome at settings of 10 and 15 µm, mounted on microscope slides and stained as indicated, using safranin and chlorazol black E as stain and counterstain respectively (Johansen, 1940). The other two leaf-sheath slices were placed on a modified MS (1962) medium containing 25 mg/l 2,4-D and 5 mg/l BA, using 2 g/l Gelrite as the solidifying agent, and placed in a dark growth chamber at 24±1°C. After 16 days one of the two culture dishes was removed from the growth chamber, the explant taken out and cut in half, and subsequently fixed and processed for microtome sectioning and mounting as described above. The third leaf-sheath explant was removed from culture after 35 days and processed similarly. Longitudinal sections were obtained from both cultured samples, and both a longitudinal and a cross-section were obtained from the sample which was fixed immediately after dissection.

RESULTS

Experiment 1:

Callus Induction on Leaf-Sheath Explants

Callusing was limited to the upper cut surface of those explants in which it occurred, and once it had become established to a thickness of 1-2 mm, it did not visibly grow further in any direction. The color of the calli ranged from white to brown, with several cream-colored shades in between the two extremes. White calli were rare and only grew in small areas between other types of callus, and never over the entire cut surface. These white calli seemed to grow the fastest and often formed small "tufts". Attempts to subculture them failed, since they turned brown upon being separated from the rest of the explant and developed no further. One week after being subcultured these callus "tufts" appeared dead. The remainder of the calli remained seemingly alive, even though no further growth was observed. Callus initiation rates on leaf-sheath sections were significantly higher in smooth palms than in spiny palms on 25-75 mg/l 2,4-D (Tables II-1 and II-2), and the interaction between explant type, i.e. smooth versus spiny, and treatment was significant (Table II-1). The best callus initiation rates for smooth and spiny pejibaye palm seedlings (53.8 and 25.0 % respectively) occurred on 25 mg/l 2,4-D (Tables II-2 and II-3, and Fig. II-2).

During the second week browning appeared in several cultures and became widespread and more intense during the third through eighth weeks. By the eighth week browning had stabilized, or continued to worsen only very gradually. As with callusing, browning was highest on 25 mg/l for both smooth

and spiny palms, with 63.3 and 47.6 % respectively (Tables II-2 and II-3, Fig. II-2). The difference between types, i.e., smooth versus spiny, was not significant. Differences among treatments and the treatment*type interaction were significant (Table II-4). Callus initiation was higher for smooth-stemmed palms on all treatments containing more than 0 mg/l but less than 100 mg/l 2,4-D (Fig. II-2). Browning was higher in smooth-stemmed palms at 2,4-D concentrations up to 50 mg/l, but above 50 mg/l the interaction became inverted and browning was higher was more pronounced in spiny-stemmed palms (Fig. II-2). Smooth-stemmed palms had lower discoloration rates than spiny-stemmed palms between 0 and 50 mg/l 2,4-D. Above 50 mg/l 2,4-D the interaction became more complex, because discoloration for smooth-stemmed palms was higher at 75 mg/l, but lower again at 100 mg/l 2,4-D (Fig. II-2).

If an explant became discolored, it did so after being in culture for at least 1 month. When probed with a teasing needle, discolored tissue disintegrated easily and was assumed to be dead. In contrast to the results on browning, discoloration was the least pronounced on 25 mg/l 2,4-D, with smooth palms showing 15.4 % compared with 36.0 % discoloration for spiny palms (Table II-2). Discoloration rates were highest on 75 and 100 mg/l 2,4-D (Table II-2). Differences between types and among treatments, and the interaction between the two variables, were significant (Table II-5, Fig. II-2). A comparison between discoloration and browning suggests an inverse relationship between the two responses for both smooth and spiny pejibaye seedlings, although this inverse relationship becomes less clear-cut as 2,4-D concentrations increase (Fig. II-3).

During the first two weeks in culture, the most noticeable change in the explants was their size increase. Their growth was not uniform, however, and

it was confined mostly to the treatments with 0, 25, 50, and 75 mg/l 2,4-D and 5 mg/l BA. Explants that enlarged did so by a factor of about 2-4. Fungal and bacterial contamination, which were 3 % and 8 % respectively, occurred mostly in explants taken from single seedlings, and therefore contamination was statistically significant within seedlings. Hence, contamination was mostly endogenous in certain seedlings, with little or no contamination being contributed by laboratory procedures.

Experiment 2:
Effect of Explant Orientation and Light on Callusing

Poor surface callusing resulted from this experiment, while browning levels were similar to those in Experiment 1. No significant differences were found between the two types of explant orientation, nor between light and dark incubation conditions. Interactions between the treatments were also non-significant.

Experiment 3: Histological Analysis

The longitudinal and cross-sections of the fresh leaf-sheath slice (Figure II-4), prepared after 0 days in culture, revealed well-organized cells with clearly recognizable vascular bundles. Cells were fairly uniform in size. After 16 days on a callus-inducing medium, cells had become very enlarged and irregular (Fig. II-5). A thin superficial layer of well-stained cells was seen on the 16-day-old culture, with several portruding cell groups which could have been

callus "tufts" (Fig. II-5). After 35 days in culture, cells continued to be large and irregularly shaped, and no superficial layer of darkly-stained cells was visible, indicating a lack of meristematic activity (Fig. II-5).

DISCUSSION AND CONCLUSIONS

Callus formation begins quickly on the upper cut surfaces of pejibaye leaf-sheath slices, since callus initiation is observed as early as a week from the establishment of the original culture. This type of callusing is characterized by an elongation and deformation of cells, rather than by an induction of rapid cell multiplication, as indicated by the lack of meristematic tissue, especially in the 35-day-old culture (Fig. II-5). This type of callus is undesirable and may explain the lack of any type of vigorous or organized growth, even after 5 months of culture. Leaf-sheath tissue does not appear to be an ideal explant for pejibaye tissue culture, unless a change in some variable of the culture medium would lead to a change in the response of the leaf-sheath explants.

Important differences between treatments were expected, since the original purpose of the experiment was to test a wide range of 2,4-D concentrations and determine which concentration worked best for callus induction. It is interesting that callus induction was optimal at 25 mg/l 2,4-D, because some references in the palm literature indicate that higher 2,4-D concentrations are often needed to induce optimum callus formation in a variety of tissues (Tisserat, 1981; Thomas and Rao, 1985; Brackpool et al., 1986). For the pejibaye palm, however, Arias and Huete (1983) had already determined that 25 mg/l 2,4-D was a good callus-inducing medium for shoot tips.

The differences in callusing and discoloration between smooth-stemmed and spiny-stemmed palms raise the question of whether traits which lead to better callusing or a higher degree of discoloration are linked to the traits of

spininess or smoothness of stem. Perhaps these differences relate to evolutionary differences of the pejibaye palm observed west and east of the Andes. Species growing west and northwest of the Andes, ranging into Central America, tend to have hard, very spiny stems, and more offshoots. Those native to areas east of the Andes and extending south to the Amazon Basin, have softer stems with fewer offshoots, and a corkier, more resistant epidermis, an adaptation which may counterbalance the reduced number of spines (Mora-Urpí, 1983).

It is difficult to explain the similar behavior of callusing and browning on the same media, since browning is generally thought to affect tissue cultures negatively (Tisserat, 1981). Perhaps wounding, which is believed to lead to the release of phenols contained in plant cells, and thereby to browning (Debergh, 1983; Kyte, 1983; and Rhodes and Wooltorton, 1978), also induces a response in plant tissue cultures which enhances callusing, as is the case with whole plants (Hartmann and Kester, 1983). From the data obtained here it appears that browning and callusing are not mutually exclusive.

In contrast, browning and discoloration <u>are</u> mutually exclusive. They could not have been recorded in any other way, of course, because their characterizing color patterns are opposed. However, if discoloration is a sign of death in the pejibaye palm explants, browning may be a sign that the tissue is still alive, however undesirable a sign of life it is, since browning can eventually lead to death or complete inactivity of the explants (Tisserat, 1981).

Callusing in most cultures of Experiment 2 was below the level expected. It is not clear what caused such insufficient callusing, especially in the cultures grown under dark conditions, since the explants were treated no differently from the explants in experiments 1 and 3.

REFERENCES CITED

Ahée, J., P. Arthuis, G. Cas, Y. Duval, G. Guénin, J. Hanower, P. Hanower, D. Lievoux, C. Lioret, B. Malaurie, C. Pannetier, D. Raillot, C. Varéchon, and L. Zuckerman. 1981. La multiplication végétative in vitro du palmier à huile par embryogenèse somatique (Vegetative propagation of the oil palm in vitro by somatic embryogenesis). Oléagineux 36(3):113-118.

Ammar, Saïda, and Abdellatif Benbadis. 1977. Multiplication végétative du palmier-dattier (Phoenix dactylifera L.) par la culture de tissus de jeunes plantes issues de semis. Compt. Rend. Acad. Sci. Paris, Sér. D 284(18): 1789-1792.

Arias M., Oscar, and Fernando Huete V. 1983. Propagación vegetativa in vitro de pejibaye (Bactris gasipaes H.B.K.). Turrialba 33(2):103-108.

Brackpool, Avril L., Richard L. Branton, and Jennet Blake. 1986. Regeneration in palms. Pages 207-222 in Vasil, Indra K., ed. Cell culture and somatic cell genetics of plants. Vol. 3. Academic Press, Inc., New York.

Branton, R. L., and Jennet Blake. 1983. Development of organized structures in callus derived from explants of Cocos nucifera L. Ann. Bot. 52(5):673-678.

Debergh, P. C. 1983. Effects of agar brand and concentration on the tissue culture medium. Physiol. Plant. 59:270-276.

Eeuwens, C. J. 1978. Effects of organic nutrients and hormones on growth and development of tissue explants from coconut (Cocos nucifera) and date (Phoenix dactylifera) palms cultured in vitro. Physiol. Plant. 42(2):173-178.

Hartmann, Hudson T., and Dale E. Kester. 1983. Plant propagation. 4th edition. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. 727 pp.

Johansen, Donald Alexander. 1940. Plant microtechnique. McGraw-Hill, New York. 523 pp.

Jones, L. H. 1974. Propagation of clonal oil palms by tissue culture. Oil Palm News 17:1-8.

Krikorian, A. D., and Robert P. Kann. 1986. Oil palm improvement via tissue culture. Pages 175-202 in Janick, Jules, ed. Plant breeding reviews. Vol. 4. Avi Publishing Company, Inc. Westport, Connecticut.

Kyte, L. 1983. Plants from test tubes. Timber Press, Portland, Oregon. 132 pp.

Mora Urpí, Jorge. 1983. El pejibaye (Bactris gasipaes H.B.K.): Origen, biología floral y manejo agronómico. Pages 118-160 in Palmeras poco utilizadas de América tropical. Reunión de consulta. (Proceedings of the workshop on underutilized palms of tropical America, 1983, Turrialba, Costa Rica). FAO. Vol. 43. FAO/CATIE, San José, Costa Rica.

Murashige, Toshio and Folke Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15(3):473-497.

Rhodes, J. M., and L. S. C. Wooltorton. 1978. The biosynthesis of phenolic compounds in wounded plant storage tissues. Pages 243-308 in Kahl, G., ed. Biochemistry of wounded plants. W. de Gruyter & Co., New York, NY.

Thomas, V., and P. S. Rao. 1985. In vitro propagation of oil palm (Elaeis guineensis Jacq var Tenera) through somatic embryogenesis in leaf-derived callus. Current Science 54(4):184-185.

Tisserat, Brent. 1979. Propagation of date palm (Phoenix dactylifera L.) in vitro. J. Exp. Bot. 30(119):1275-1283.

Tisserat, Brent. 1981. Date palm tissue culture. U.S. Department of Agriculture. Agricultural Research Service. Advances in Agricultural Technology. Western Series. No. 17. (U.S. Department of Agriculture, Oakland, Calif.). 50 pp.

Tisserat, Brent. 1984. Clonal propagation: Palms. Pages 74-81 in Vasil, Indra K., ed. Cell culture and somatic cell genetics of plants. Vol.1. Academic Press, Inc., New York.

Tomlinson, P. B. 1961. Palmae. Vol 2. of Metcalfe, C. R., ed. Anatomy of monocotyledons. Oxford University Press, Clarendon, England. 453 pp.

Table II-1. Analysis of variance for callusing of pejibaye leaf-sheath tissue. (Callusing was scored as being either present [1] or absent [0]. "TYPE" refers to smooth-stemmed versus spiny-stemmed palms; "GENOT" refers to genotype, i.e., differences between individual seedlings; and "TRT" refers to the BA and 2,4-D treatments used)

Source	df	Type III SS	f Value
TYPE	1	1.11	8.12**
GENOT(TYPE) (Error a)	81		
TRT	5	11.11	17.75***
TYPE*TRT	5	5.91	4.37***
TYPE*TRT*GENOT(TYPE) (Error b)	349		
CORRECTED TOTAL	441	45.11	

**Significant at the 1 % level.
***Significant at the 0.1 % level.

Table II-2. Percentages of callusing, browning, and discoloration for pejibaye leaf-sheath explants of smooth and spiny palms, as affected by BA and 2,4-D

TREATMENT		RESPONSES					
BA	2,4-D	CALLUSING		BROWNING		DISCOLORATION	
		Smooth	Spiny	Smooth	Spiny	Smooth	Spiny
0	0	0.0	0.0	53.8	31.7	27.5	63.4
5	0	7.9	8.0	52.9	26.9	26.3	65.4
5	25	53.8	25.0	63.3	47.6	15.4	36.0
5	50	20.0	5.1	37.8	47.5	42.5	45.0
5	75	21.6	0.0	17.6	32.5	62.1	55.0
5	100	2.4	0.0	17.1	24.4	58.5	73.2
MEANS		17.4	4.8	39.5	34.4	38.7	57.3

Table II-3. Percentages of callusing, browning, and discoloration for pejibaye leaf-sheath tissue, as affected by BA and 2,4-D

TREATMENT		RESPONSE MEANS		
BA	2,4-D (mg/l)	CALLUSING	BROWNING	DISCOLORATION
0	0	0.0	42.5	45.7
5	0	7.9	41.7	42.2
5	25	42.8	56.9	23.4
5	50	12.6	42.8	43.7
5	75	10.5	25.7	58.4
5	100	1.2	20.7	65.8

Table II-4. Analysis of variance for browning of pejibaye leaf-sheath tissue. (Browning was scored as being either present [1] or absent [0]. "TYPE" refers to smooth-stemmed versus spiny-stemmed palms; "GENOT" refers to genotype, i.e., differences between individual seedlings; and "TRT" refers to the BA and 2,4-D treatments used)

Source	df	Type III SS	f Value
TYPE	1	0.29	0.75$^{n=}$
GENOT(TYPE) (Error a)	82		
TRT	5	5.67	6.44***
TYPE*TRT	5	2.34	2.66*
TYPE*TRT*GENOT(TYPE) (Error b)	330		
CORRECTED TOTAL	423	98.86	

*Significant at the 5 % level.
***Significant at the 0.1 % level.
$^{n=}$Non-significant.

Table II-5. Analysis of variance for discoloration of pejibaye leaf-sheath tissue. (Discoloration was scored as either being present [1] or absent [0]. "TYPE" refers to smooth-stemmed versus spiny-stemmed palms; "GENOT" refers to genotype, i.e., differences between individual seedlings; and "TRT" refers to the BA and 2,4-D treatments used)

Source	df	Type III SS	f Value
TYPE	1	2.75	7.22**
GENOT(TYPE) (Error a)	82		
TRT	5	7.41	7.87***
TYPE*TRT	5	3.25	3.45**
TYPE*TRT*GENOT(TYPE) (Error b)	354		
CORRECTED TOTAL	447	111.73	

**Significant at the 1 % level
***Significant at the 0.1 % level

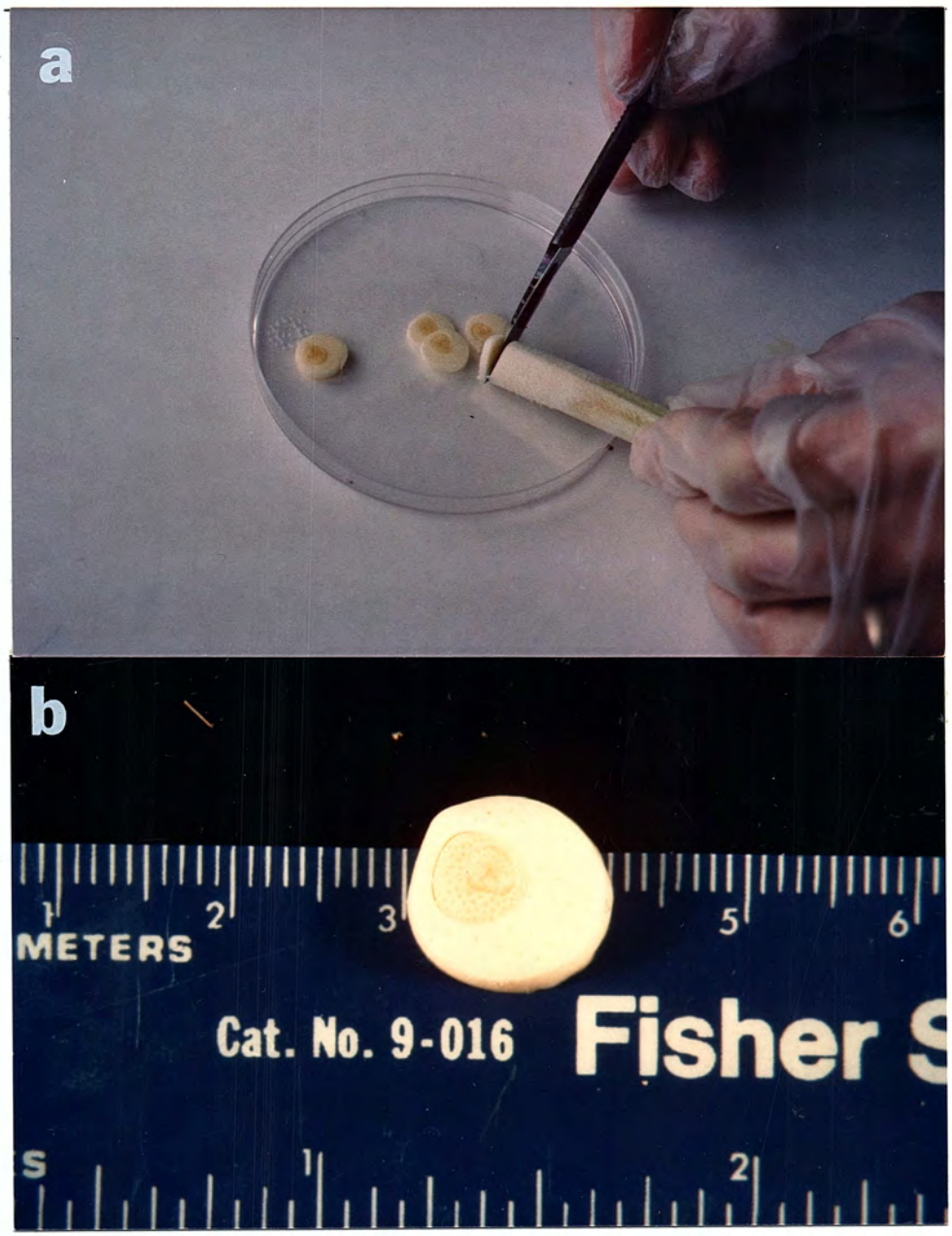


Figure II-1. Dissection of leaf-sheath explants. (a) Cutting leaf-sheath slices. (b) Closeup of a leaf-sheath explant

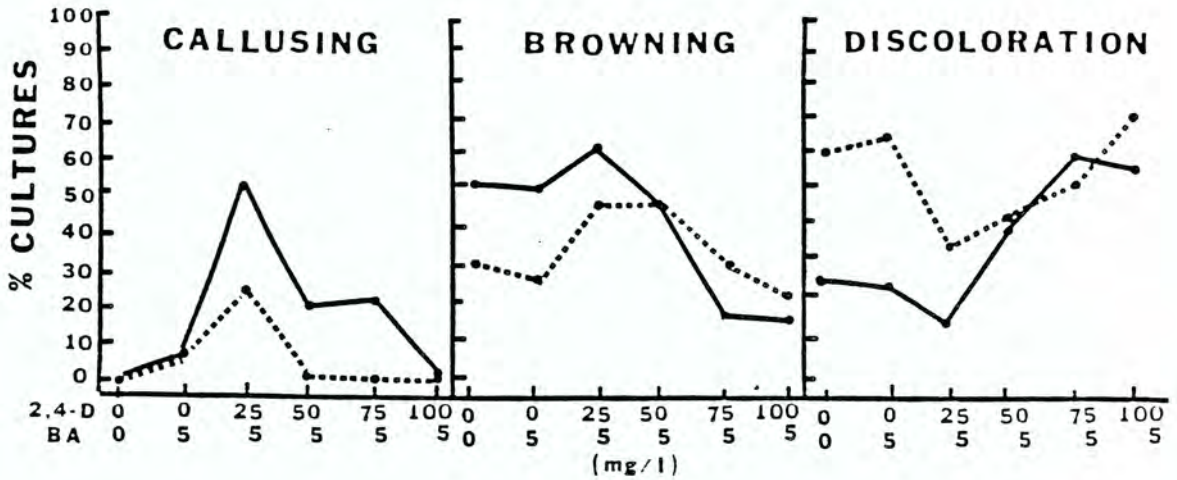
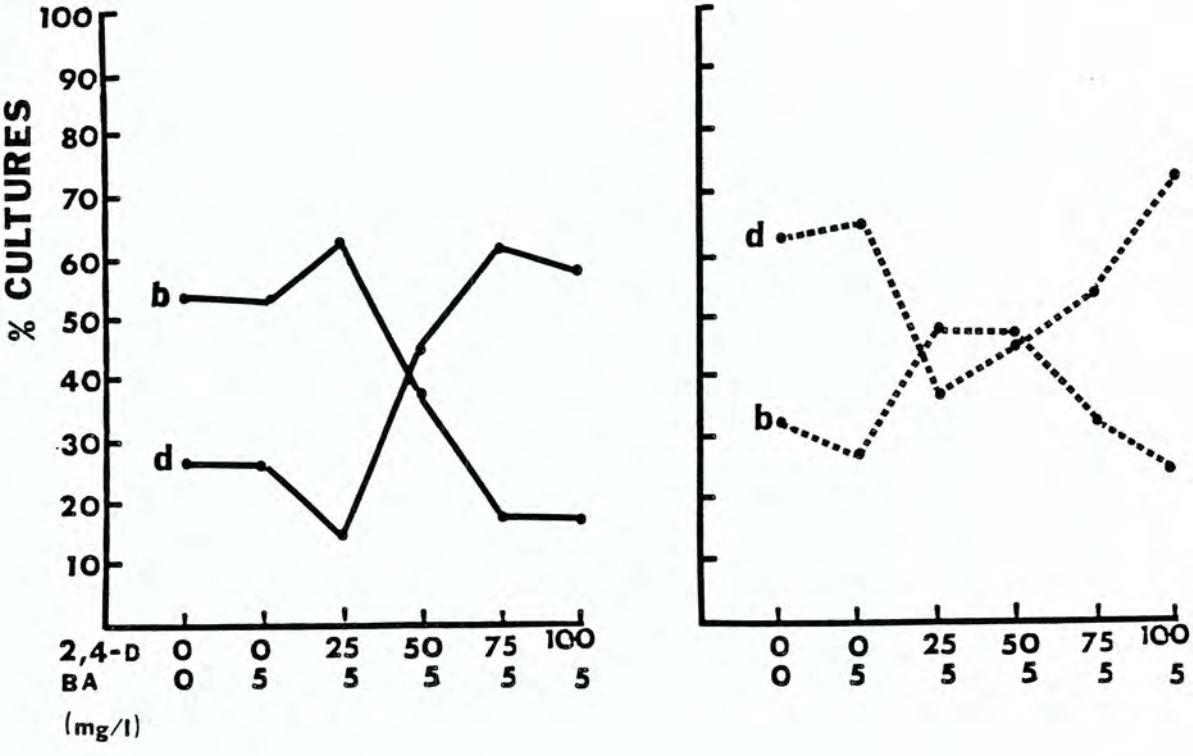
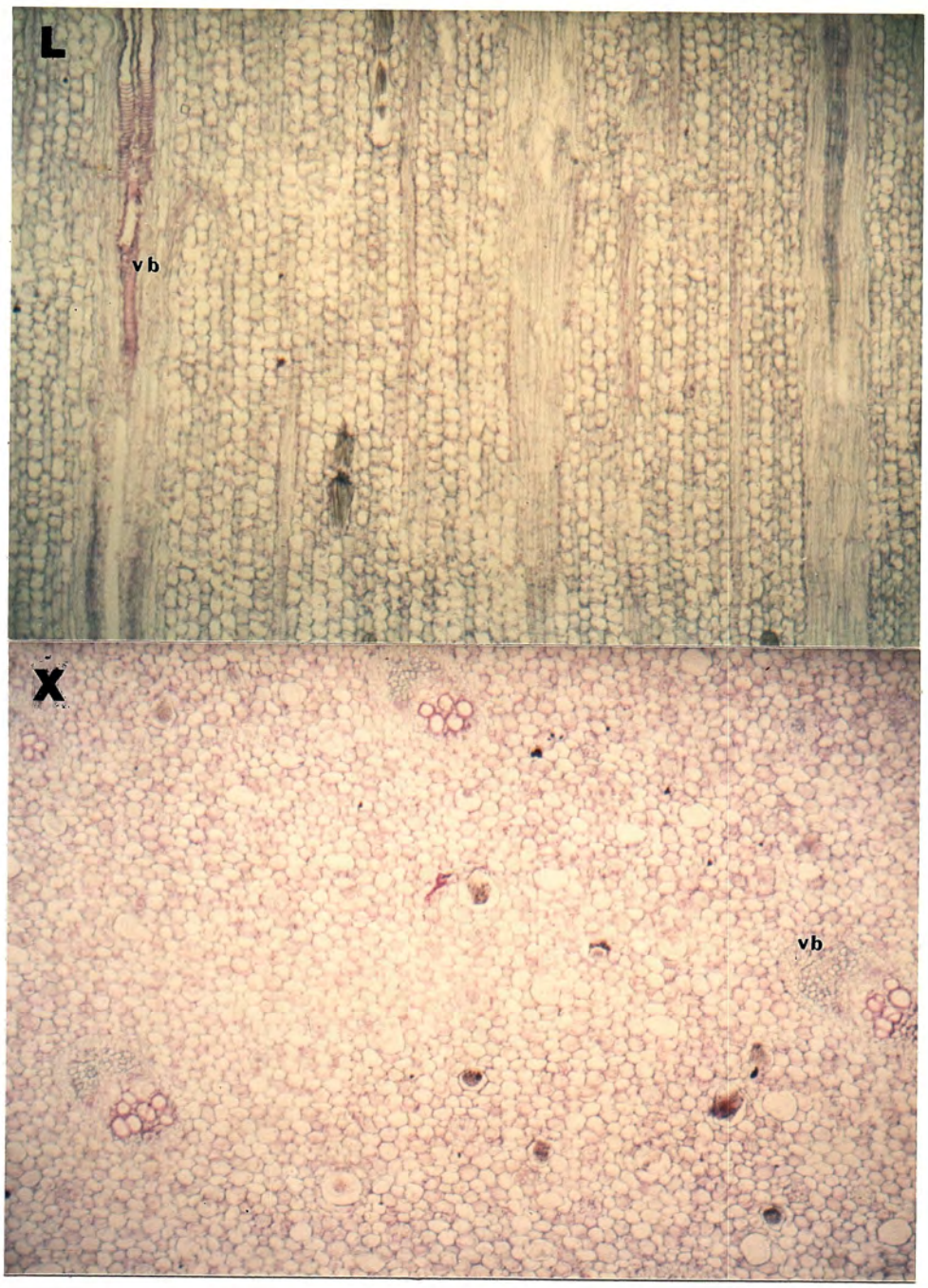


Figure II-2. Differences in callusing, browning, and discoloration rates between leaf-sheath explants of smooth-stemmed and spiny-stemmed pejibaye palm seedlings



b = browning
d = discoloration
——— = smooth-stemmed
••••••• = spiny-stemmed

Figure II-3. Relationship between browning and discoloration in leaf-sheath explants of smooth-stemmed and spiny-stemmed pejibaye seedlings cultured in vitro



vb = vascular bundles

Figure II-4. Longitudinal (L) and cross-sections (X) of a fresh pejibaye leaf-sheath slice. (Magnification: 20.5x)

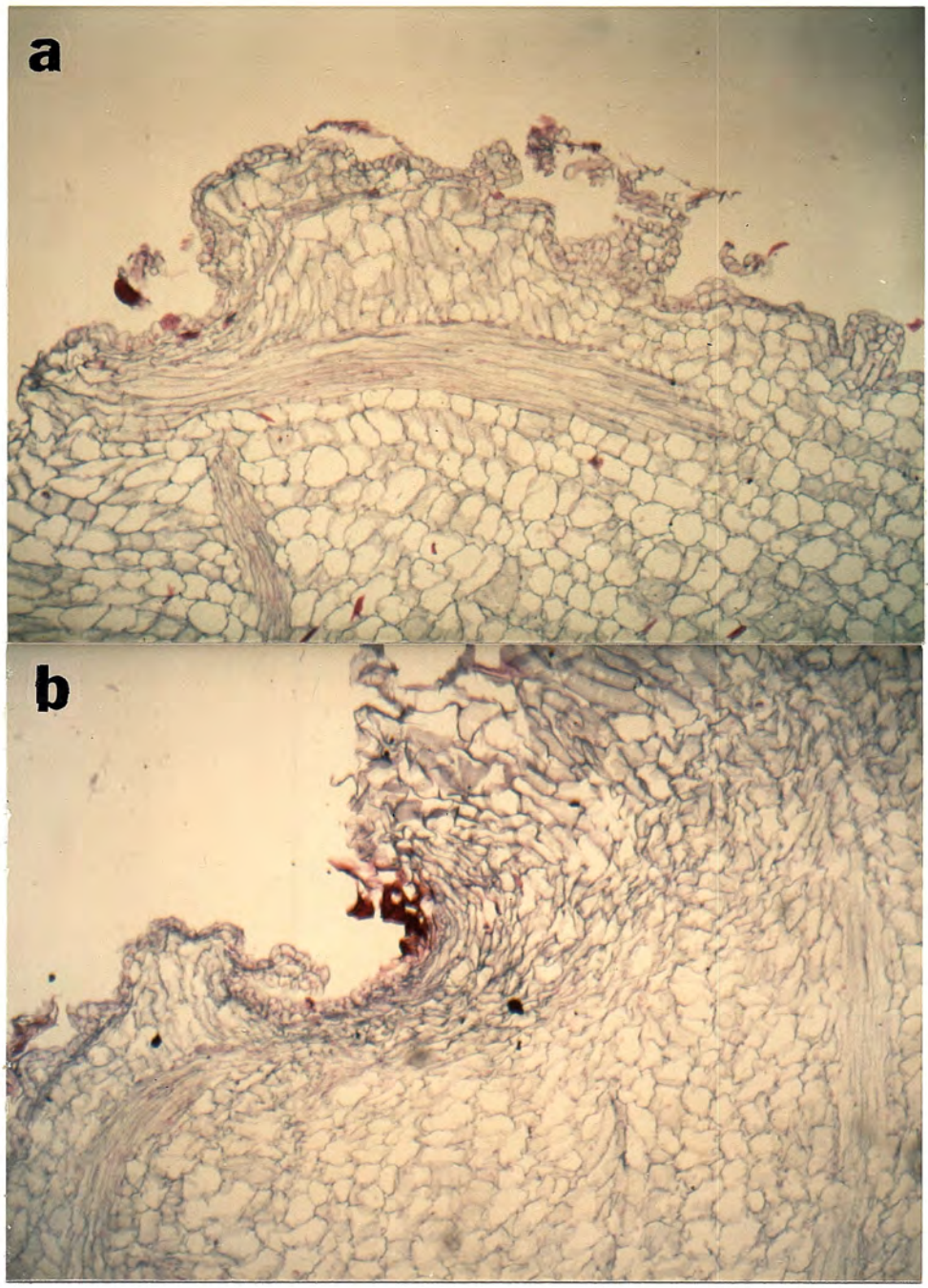


Figure II-5. Histological slides of pejibaye callus cultures.
(a) Longitudinal section of a pejibaye leaf-sheath slice after 16 days on a callus-inducing medium containing 25 mg/l 2,4-D and 5 mg/l BA. (b) Longitudinal section of a leaf-sheath slice after 35 days on a callus-inducing medium containing 25 mg/l 2,4-D and 5 mg/l BA. (Magnification: 20.5x)

PART III. THE EFFECT OF HIGH CYTOKININ:AUXIN RATIOS ON PEJIBAYE TISSUE CULTURES

ABSTRACT

Subapical stem sections, leaf-sheath sections, and shoot tips were cultured on media containing high ratios of BA:2,4-D or BA:NAA. A total of 12 growth regulator combinations were tested in each case, all added to a basal MS (1962) medium formulation. Subapical stem sections produced actively growing, superficial calli on media containing BA and 2,4-D. This was the case despite widespread browning on the cultures. Twelve percent of the cultures developed globular structures with a diameter of about 3 mm, from which single roots emerged in three cases. No significant differences among treatments were found for callusing or browning, but significant differences existed between smooth-stemmed and spiny-stemmed seedlings for callusing, with spiny-stemmed seedlings showing the highest callusing means. Leaf-sheath sections and shoot tips did not grow substantially or produce any calli or organogenic responses on the media with BA and 2,4-D.

The combinations of BA and NAA, all of which contained 2.5 g/l activated charcoal, did not induce callusing, organogenesis, or substantial growth in any of the explant types.

Histological sections of fresh subapical stem tissue revealed evenly-shaped cells, with somewhat irregular-growing vascular bundles near the root zone. A longitudinal section of a subapical stem callus showed cell groups in distinct circular arrangements on the callus surface. No bipolarity was noted in these cell groups. A cross section of a globular structure showed a root initial growing from it.

INTRODUCTION

Most studies in the in vitro culture of somatic palm tissue focus on the use of high auxin:cytokinin ratios in the medium with the purpose of inducing callus formation. Skoog and Miller (1957) determined that high cytokinin:auxin ratios typically induce the formation of shoots, rather than roots or calli. The effect of given growth-regulator ratios in culture media on the in vitro response of the explants is not clear-cut, however. Skoog and Miller (1957) implied that endogenous levels of auxins and cytokinins control the development of the various organ types, and many studies have confirmed this (Halperin, 1986). "[It] is not unusual to find cultured tissues in which the exogenous auxin/cytokinin ratio either exerts no control over the type of organ regenerated or has an effect opposite to that observed by Miller and Skoog (1957)" (Halperin, 1986). In line with this statement, Smith and Thomas (1973) found that cytokinin inhibited callus formation in isolated Elaeis guineensis cells. However, at least one source reports callus formation on coconut stem tissue using 10^{-7} M 2,4-D, 5 x 10^{-6} M BA, and coconut water (Apavatjrut and Blake, 1977). Pinedo Panduro (1987) utilized high BA:NAA ratios to induce direct organogenesis in pejibaye shoot tips.

Vegetative shoots may originate in several different ways in the pejibaye palm. Offshoots develop from axillary buds at the base of the stipe, and also from root promeristems (J. Mora-Urpí, Dept. of Biology, Univ. of Costa Rica, San José, Costa Rica; personal communication, 1987). Vegetative offshoots occasionally develop from axillary buds in the aerial portion of the plant (Mora-Urpí, personal communication, 1987). Of direct relevance to this experiment is the occurrence of vegetative shoots in the scars left on stems when a frond falls

off, and the rare occurrence of shoots in the center of trunks which have been left standing in the field after the top portion was removed. In the former instance, the shoots form a ring following the outer contour of the stem, and are suspected to emerge from the severed ends of vàscular bundles. In the latter case, the origin of the shoots may also be vascular bundles whose parenchymatous cells have differentiated (Mora-Urpí, personal communication, 1987). The primary purpose of this experiment was to test the possibility of inducing organogenesis from vascular bundles in pejibaye stem tissue in vitro, although leaf-sheath explants and shoot tips were included for comparison.

MATERIALS AND METHODS

Experiment 1:

Pejibaye Tissue Culture on Media Containing High Cytokinin:Auxin Ratios

Two split-plot designs were set up, one with Media Set A, the other with media Set B (see Table III-1). Each media set contained twelve different media preparations. As illustrated in Fig. III-1, most media in each set were distributed over a central range of growth regulator concentrations, where the highest level of tissue culture activity was expected, and a few combinations in each set tested responses at extremes. Set A contained combinations of 2,4-D and BA without any activated charcoal, whereas in Set B, combinations of NAA and BA were used, and 2.5 g/l activated charcoal were added to all treatments. The basal medium used in all cases was a modified MS (1962) formulation containing 2 g/l Gelrite. After the pH had been adjusted to 5.8, the media preparations were autoclaved for 15 minutes at 1.1 kg/cm^2 and 121°C, and then dispensed into disposable 6-cm petri dishes in 15-ml aliquots. One half of each split-plot design tested the responses of smooth-stemmed pejibaye palm seedlings, the other half tested spiny-stemmed seedlings. There were three replications per treatment per seedling type.

The seedlings were imported from Costa Rica when they had just emerged from the seeds, and were grown in individual pots in a greenhouse at 27°C. No artificial light was used. The seedlings were watered every other day and fertilized every other week with a soluble greenhouse fertilizer containing

200 ppm N. Iron chelate was added to the fertilizer if chlorosis symptoms appeared.

Explants were obtained by cutting off the roots, foliage, and part of the stem of the palm seedlings, until 9-cm stem segments were left which included leaf-sheath tissue and the broad stem base. These segments were rinsed several times to remove the soil. The outermost tissue layer, i.e., the base of a leaf sheath, of each stem segment was peeled off, and they were subsequently surface sterilized by immersing them in 70 % ethyl alcohol for one minute. This step was followed by a transfer of the stem segments into a 30 % (v/v) chlorine bleach solution containing 2 drops of the surfactant "Tween 80", in which they were shaken continuously for 15 minutes.

Upon completion of this step the stem segments were rinsed 3-4 times with sterile deionized water under aseptic conditions. Two more tissue layers, or leaf sheaths, were then removed from these segments, which left leaf-sheath cylinders formed by the inner two or three layers of stem tissue encapsuling leaf primordia, with the broad stem base still attached at the proximal end. To avoid contamination of explants during the dissection procedure, the distal end of the stem segment, where the foliage had been attached, was held by hand outside the petri dish in which the dissection was carried out, while the proximal end rested inside the petri dish. Subapical stem tissue was obtained from the broad stem base by cutting thin slices (1 mm or less in thickness) from the proximal end (Fig. III-2), after the possibly bleach-damaged surface tissue of the broad stem base had been carefully trimmed off.

To obtain shoot tips, thin slices were cut from the broad stem base until several concentric "rings" became visible in the broad stem base tissue.

This indicated that the cuts had reached an area very close to the shoot tip, which contains the apical meristem. At this point a circle was cut into the broad stem base tissue following the contour of the innermost "ring" visible. By squeezing the base of the stem section gently between two fingers, the shoot tip, with a thin layer (1-2 mm) of broad stem base tissue still attached at its base, was ejected from the stem section without suffering any visible damage (See PART IV for an illustration of the shoot tip extraction). At this point a shoot tip was ready to be placed on the medium.

Leaf-sheath sections were obtained by removing the rest of the broad stem base tissue and the outermost leaf sheath. This leaf sheath had been squeezed between the fingers and was therefore expected to be contaminated. Two-millimeter slices were cut from the proximal end of the remaining leaf-sheath cylinder (for a pictorial illustration see PART II). The cultures of each media set were placed in a dark growth chamber for three months, and then transferred to light under 1600 lux, 18 hours a day. The dark and lighted growth chambers were both kept at 24±1°C. Explants were subcultured once a month, and final observations were made after 5 months. Due to a high incidence of contamination, Type III Sums of Squares were utilized in the analysis of variance. Callusing was evaluated on a subjective scale where 0 stood for no callusing, 1 for some callusing on 25 % or less of the explant surface, 2 for active callusing over 25-50 % of the explant, and 3 for active callusing over more than 50 % of the upper cut surface. Browning was scored as 0 (no browning), 1 (light browning on the bottom and along the edges of the explant), 2 (dark browning on the bottom, along the edges, and on parts of the upper surface of the explant), and 3 (complete browning).

Experiment 2: Histological Analysis

A subapical stem slice was dissected as described in Experiment 1 and fixed immediately, then dehydrated and embedded in paraffin, after Johansen (1940). Tissue slices were cut on a microtome at settings of 10 and 15 µm, mounted on microscope slides and stained as indicated using safranin and chlorazol black E as stain and counterstain respectively (Johansen, 1940). The same histological process was followed for one explant which was chosen arbitrarily for its good callus formation after five months in culture, and another which was chosen for the globular structures it had developed in culture.

RESULTS

Experiment 1:

Pejibaye Tissue Culture on Media Containing High Cytokinin:Auxin Ratios

On media Set A, subapical stem explants started to form calli on the upper cut surface, analogous to the leaf-sheath sections in PART II, after two weeks in culture. The calli were white and had a frosty appearance (Fig. III-3), and most continued to grow despite browning along the edges and the bottom of the explants. Some cultures were discarded due to complete browning or lack of response, and others were discarded due to contamination, which was high in comparison with the other studies reported in this thesis. Contamination consisted of 15 % bacterial contamination and 13 % fungal contamination.

Callus formation on the subapical stem slices was notably more vigorous than the callus formation on leaf-sheath tissue observed in PART II. At the end of three months, globular callus formations (Figure III-4), from which single roots emerged in a few instances, were observed in 12 % of the cultures. These globular calli occurred on media with 0.5, 4.0, 6.0, 8.0, and 10.0 mg/l BA, and 0.1, 3.0, 1.0, 5.0, and 9.0 mg/l 2,4-D respectively. No significant differences were observed among treatments for callus initiation. Significant differences in callusing were detected between spiny-stemmed and smooth-stemmed seedlings, however (Table III-2), with spiny-stemmed seedlings producing a callusing mean of 1.63, versus 1.14 in smooth genotypes.

Callusing means for all treatments are listed in Table III-3. No significant differences for browning were found among treatments or between

smooth-stemmed and spiny-stemmed seedlings. Shoot tips and leaf-sheath explants on the same media set (Set A, with BA and 2,4-D) failed to give any response beyond some 2-fold enlargement during the first two weeks in culture. Most leaf-sheath slices had browned severely after two months and were discarded together with the shoot tips at the end of four months. Some bacterial and fungal contamination was observed, although it was not observed on more than 5 % of the shoot-tip or leaf-sheath cultures.

The only responses in the cultures on media Set B, which contained BA and NAA, were stem slice expansion and some shoot-tip elongation, with tissue greening after the cultures were placed in the light. Enlargement was not more than twice the original size in any of these cultures, and most of the growth occurred during the first three weeks of culture. Browning became severe after the first two months and eventually covered most stem and leaf-sheath explants, but only a few shoot-tip explants. No callusing was observed except on one subapical stem explant, which eventually turned completely brown. All cultures on media Set B were discarded after four months.

Experiment 2: Histological Analysis

The longitudinal and cross sections of the fresh subapical stem segment (Fig. III-5) show organized cells, with vascular bundles less regularly arranged than in leaf-sheath sections (compare with Experiment 2, PART II). The longitudinal callus section (Fig. III-6) shows enlarged cells with a layer of

well-stained cells and a globular cell arrangement on the upper surface. The cross section of the globular structure (Fig. III-7) contains what may be a root initial growing out of the globule.

DISCUSSION AND CONCLUSIONS

Since an organogenic response and no callusing were expected, it was surprising that high cytokinin:auxin ratios on media Set A, with BA and 2,4-D, gave rise to fairly vigorous callus growth on subapical stem sections, and that they did so over a wide range of concentrations (Tables III-1 and III-3). Even though there were no significant differences in callusing between the treatments, the use of BA concentrations between 4.0 and 8.0 mg/l, in combination with 0.0-7.0 mg/l 2,4-D and no coconut water, is recommended if callusing is desired, since the highest callusing means were observed on treatments with those concentration ranges of BA and 2,4-D (Table III-3).

The only organogenic response observed was the formation of roots on three of the seven cultures which produced globular structures. Since the subapical stem tissue is so close to the root zone in palm seedlings, it may contain meristematic root primordia which make this type of tissue quite responsive to in vitro conditions. This provides hope that pejibaye stem tissue can be used to propagate the palm. The results in this experiment suggest that endogenous, rather than exogenous, cytokinin:auxin ratios determine the in vitro response of pejibaye stem tissue, because calli and roots were produced on high cytokinin:auxin ratios instead of shoots. In fact, the exogenous growth regulators, i.e. BA and 2,4-D, may not have affected the in vitro response of the pejibaye stem explants at all, or affected it in a way contrary to what was expected, as Halperin (1986) suggested.

The advantage of stem tissue over shoot tips is that several sections can be obtained from a single stem, whereas only one shoot tip is present per

seedling. The disadvantage that subapical stem tissue has, however, is that it grows close to the soil in seedlings and hence appears to be more prone to contamination than leaf-sheath tissue or shoot tips. Contamination of subapical stem tissue may not be a severe problem in subapical stem tissue taken from adult plants, since in their case the stem tissue extends away from the soil. Both the contamination problem and further tissue culture responses of subapical stem explants demand further attention.

Although leaf-sheath tissue has distinct vascular bundles, which, similar to the vascular bundles in a few of the adult plants observed in the field in Costa Rica, might be induced to produce shoots under in vitro conditions, no such response was observed here. A conclusion that obtaining such a response in vitro is impossible cannot be drawn yet, because there may be only a few plants capable of doing so due to their genotype, and it is impossible to determine whether such a genotype was present in this study. However, taking into account the results of PART II, it appears that leaf-sheath slices are not very responsive to tissue culture manipulation.

Several reasons can be offered for why shoot tips failed to develop on any of the treatments, even though high cytokinin:auxin ratios usually encourage morphological development and the shoot tips had been expected to grow. It is possible that none of the growth regulator concentrations in this experiment were conducive to shoot development. This hypothesis is supported by the results in PART IV, in which shoot-tip elongation occurred mostly on media containing activated charcoal and coconut water, without any growth regulators. It is also possible that a combination of BA and NAA without any activated charcoal in the

medium would have resulted in shoot-tip development, as suggested by Pinedo Panduro's results (1987).

The fact that no satisfactory results were obtained on any of the cultures on media Set B, which contained BA, NAA, and activated charcoal, may be attributed to the adsorptive effect of activated charcoal, which can render growth regulators ineffective (Weatherhead et al., 1978).

REFERENCES CITED

Apavatjrut, Pimchai, and J. Blake. 1977. Tissue culture of stem explants of coconut (Cocos nucifera L.). Oléagineux 32(6):267-270.

Johansen, Donald Alexander. 1940. Plant microtechnique. McGraw-Hill, New York. 523 pp.

Murashige, Toshio and Folke Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15(3):473-497.

Pinedo Panduro, Mario Herman. 1987. Organogénesis directa en ápices caulinares de pejibaye (Bactris gasipaes H.B.K.). Master's Thesis. Centro Agronómico Tropical de Investigación y Enseñanza, Turrialba, Costa Rica. 110 pp.

Salazar, Sebastián S. 1986. The effect of 2,4-D, light, and activated charcoal on offshoot-tip culture of the pejibaye palm (Bactris gasipaes H.B.K.). Master's Thesis. University of Birmingham, Birmingham, England. 70 pp.

Skoog, Folke, and Carlos O. Miller. 1957. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp. Soc. Exp. Biol. 11:118-131.

Smith, W. K., and J. A. Thomas. 1973. The isolation and in vitro cultivation of cells of Elaeis guineensis. Oléagineux 28(3):123-127.

Weatherhead, M. A., J. Burdon, and G. G. Henshaw. 1979. Effects of activated charcoal as an additive plant tissue culture media: Part 2. Z. Pflanzenphysiol. 94(5):399-405.

Table III-1. Media combinations of the two media sets utilized in Experiment 1. ("COC" stands for coconut water, "T" stands for treatment)

SET A:	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
BA (mg/l)	--	0.5	4	4	6	6	8	8	10	12	12	24
2,4-D (mg/l)	--	0.1	3	7	--	1	5	5	9	3	7	18
COC (ml/l)	100	--	100	--	--	--	--	100	--	--	100	--

SET B:	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
BA (mg/l)	--	--	--	5	5	10	10	10	10	15	15	20
NAA (mg/l)	--	2	2	1	3	--	2	2	4	1	32	20
COC (ml/l)	100	--	100	--	--	--	--	100	--	--	---	--

Table III-2. Analysis of variance for callusing on subapical pejibaye stem explants. ("TYPE" refers to smooth versus spiny-stemmed explants, "GENOT" refers to the difference between individual seedlings, and "TRT" stands for treatment)

Source	df	Type III SS	f Value
TYPE	1	2.65	4.29*
GENOT(TYPE) (Error a)	17		
TRT	11	15.76	1.88^{ns}
TYPE*TRT	10	6.40	0.84^{ns}
TYPE*TRT*GENOT(TYPE) (Error b)	7		
CORRECTED TOTAL	47	60.67	

*Significant at the 5 % level.
^{ns}Non-significant.

Table III-3. Callusing means for subapical stem explants taken from smooth-stemmed and spiny-stemmed pejibaye palm seedlings. (Callusing was scored as 0 = no callusing, 1 = some callusing over 25 % or less of the explant surface, 2 = active callusing over 25-50 % of the explant, and 3 = active callusing on at least 50 % of the explant)

	TREATMENT											
SET A:	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
BA (mg/l)	--	0.5	4	4	6	6	8	8	10	12	12	24
2,4-D (mg/l)	--	0.1	3	7	--	1	5	5	9	3	7	18
COC (ml/l)	100	--	100	--	--	--	--	100	--	--	100	--
MEANS												
SMOOTH	0.7	1.3	0.7	1.5	2.0	3.0	2.5	1.0	1.3	0.0	0.3	0.0
SPINY	0.0	1.5	2.0	3.0	1.0	3.0	2.5	3.0	0.5	2.0	2.0	0.0
TREATMENT MEAN	0.3	1.4	1.3	2.2	1.5	3.0	2.5	2.0	1.7	1.0	1.1	0.0

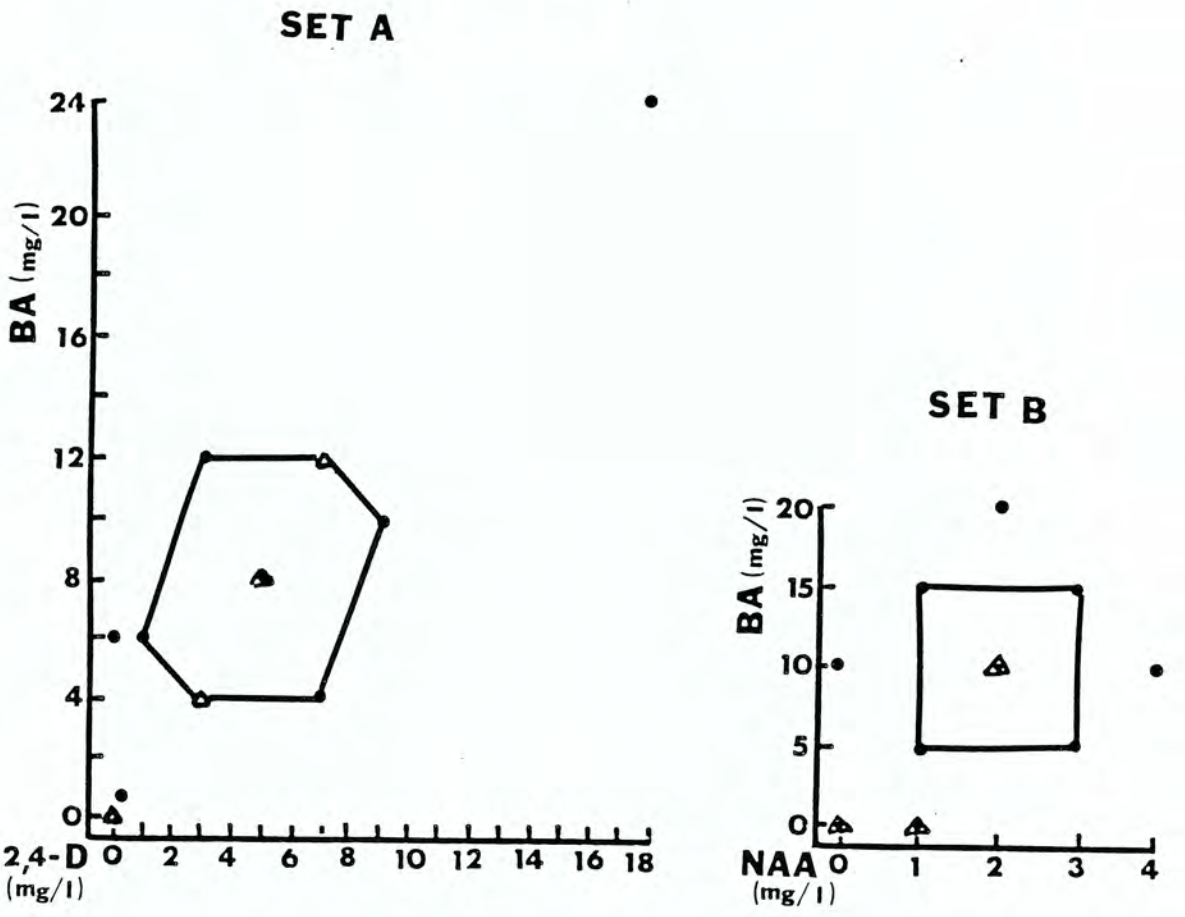
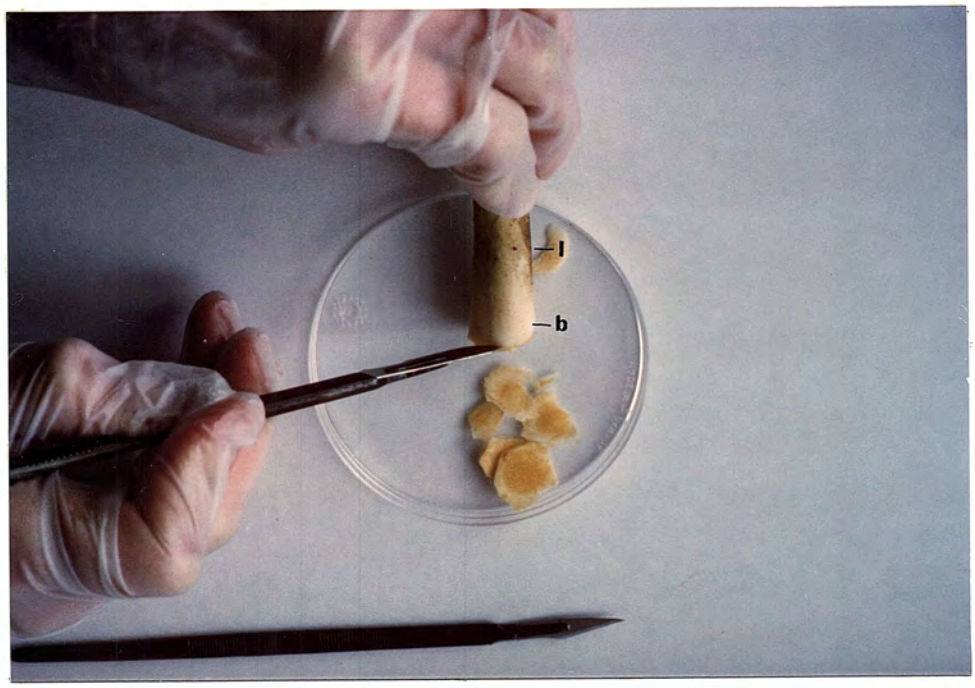


Figure III-1. Media distributions in Media Set A and Media Set B. Most of the growth regulator combinations in each set are distributed along a range that was expected to yield the most active in vitro response. A few medium combinations are outside the central ranges in order to test responses at the extremes. Media indicated by a triangle contained 100 ml/l coconut water



b = broad stem base
l = leaf-sheath tissue

Figure III-2. Dissection of thin subapical stem slices from the broad stem base of a pejibaye seedling

Figure III-3. An example of active callusing on the upper cut surface of a subapical stem explant after 3 months on a medium containing 8.0 mg/l BA, 5.0 mg/l 2,4-D, and 100 ml/l coconut milk. The diameter of the explant shown here is 1.5 cm

Figure III-4. Globular callus formations on a subapical pejibaye stem explant after 3 months on a medium containing 6.0 mg/l BA and 1.0 mg/l 2,4-D. The diameter of the explant shown here is 2 cm

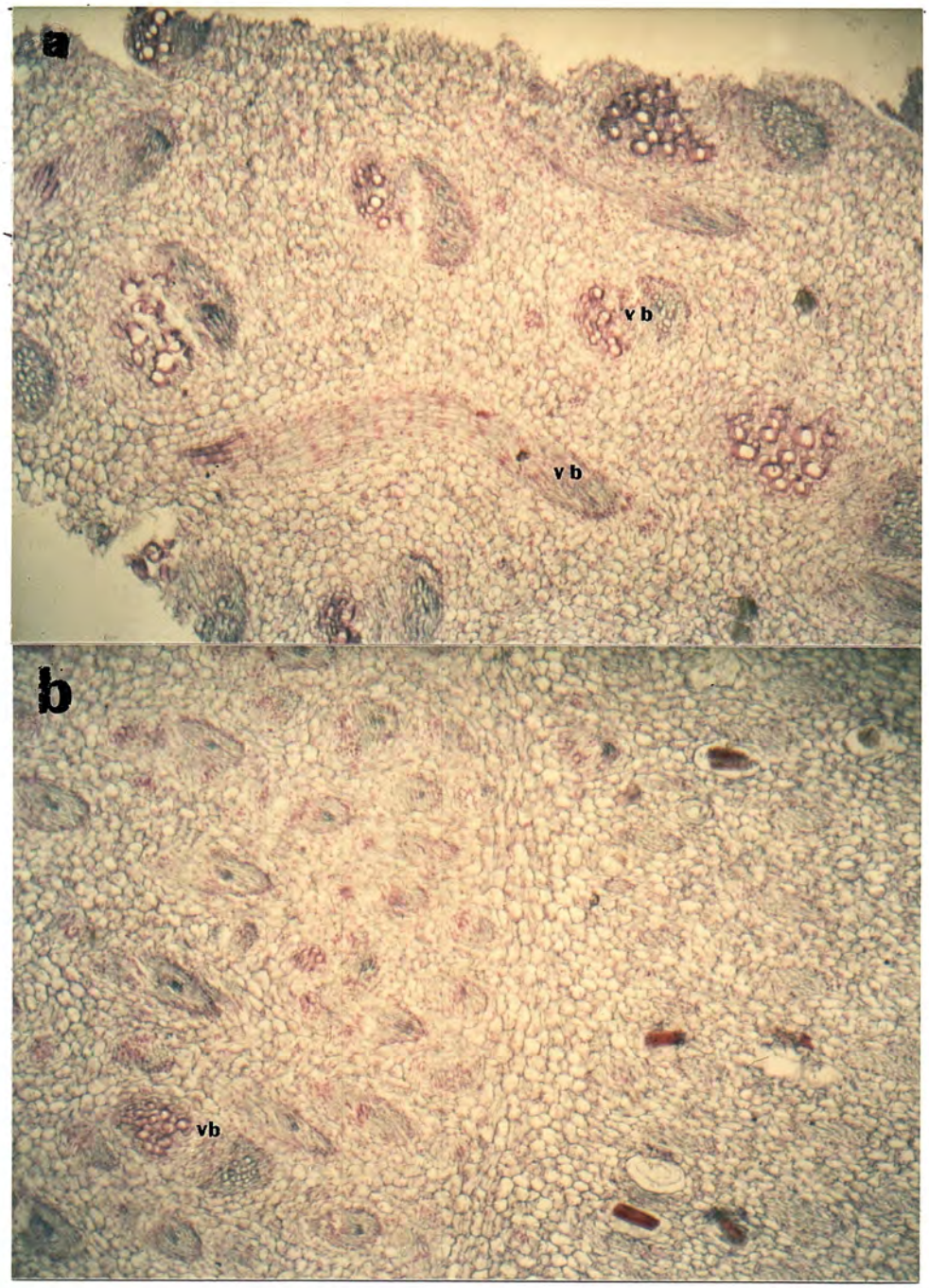


Figure III-5. Histological sections of pejibaye stem tissue. (a) Longitudinal section of a subapical pejibaye stem slice near the root zone showing irregularly arranged vascular bundles (vb). (b) Cross section of a subapical stem slice near the shoot tip. (Magnification: 20.5x)

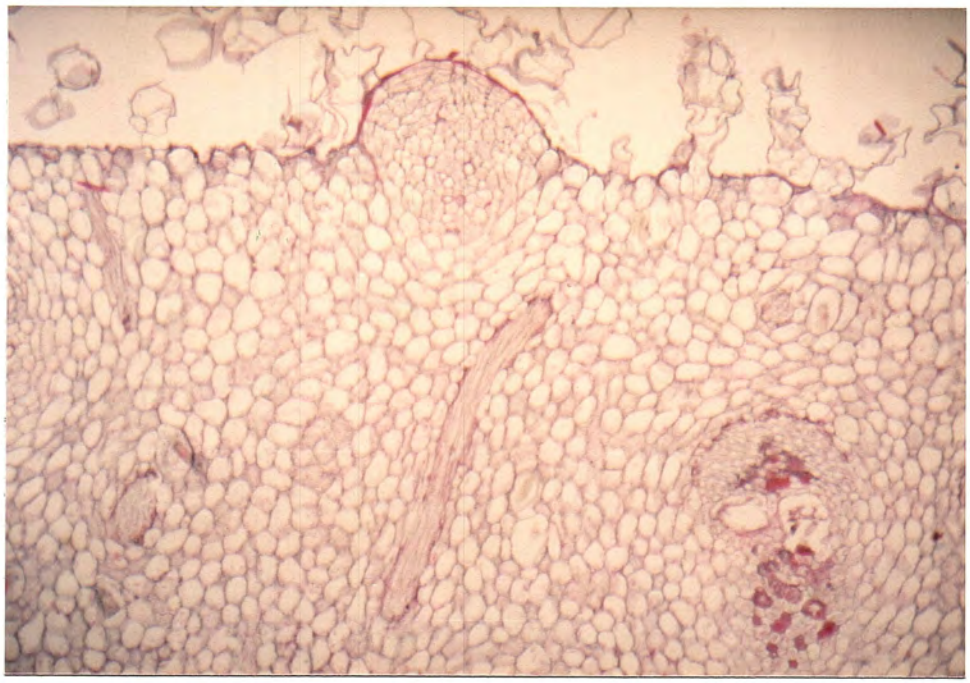
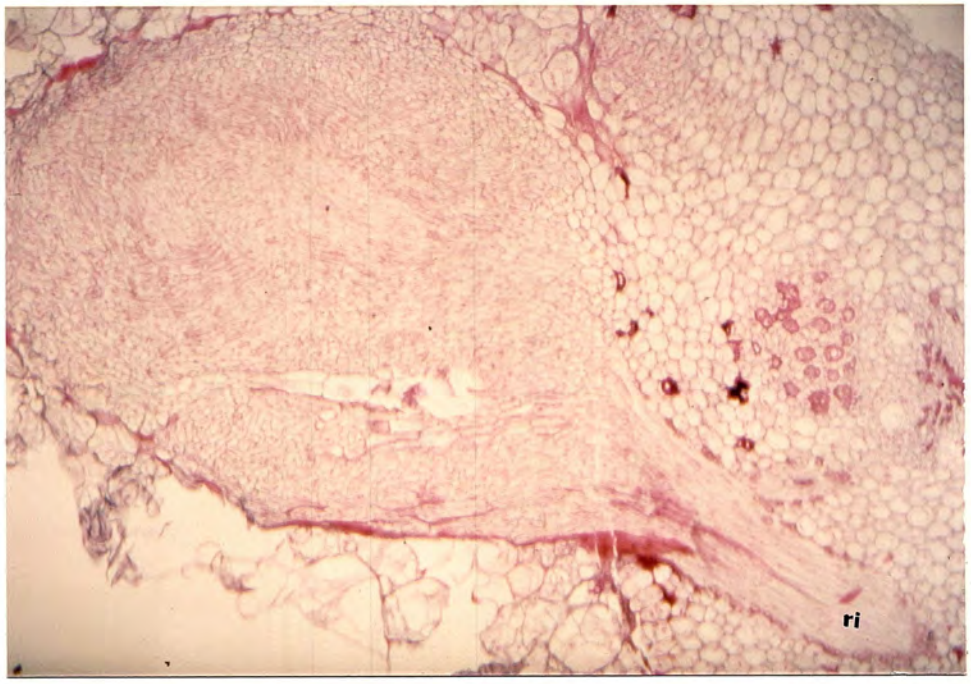


Figure III-6. Longitudinal section of a pejibaye stem callus with distinct cell groupings on the surface. The culture medium contained 6.0 mg/l BA and 1.0 mg/l 2,4-D. (Magnification: 20.5x)



ri = root initial

Figure III-7. Cross section of a globular callus structure showing a root initial growing from it. The culture medium contained 8.0 mg/l BA, 5.0 mg/l 2,4-D, and 100 mg/l coconut water. (Magnification: 20.5x)

PART IV. THE EFFECTS OF ACTIVATED CHARCOAL, COCONUT WATER, AND PLANT AGE ON THE TISSUE CULTURE OF PEJIBAYE SHOOT TIPS

ABSTRACT

Shoot tips from 1½-year-old and 4-month-old seedlings were placed on MS media containing 0, 25, 50, 75, and 100 mg/l 2,4-D, with 5 mg/l BA in all but the control treatments, which contained no 2,4-D either. Coconut water and activated charcoal were added in various combinations, or left out completely. Callus production was most vigorous on 25 mg/l 2,4-D without coconut water or activated charcoal, and significantly more so for 4-month-old seedlings than for 1½-year-old seedlings. Calli were completely absent from media containing activated charcoal at 2,4-D concentrations below 50 mg/l. Organogenic responses were observed predominantly on media containing activated charcoal and high 2,4-D levels, or no activated charcoal and low 2,4-D levels. Organogenesis occurred without an intermediary callus stage. Some of the media produced both calli and organogenesis, sometimes even within one culture. Death of explants was manifested by discoloration of the tissue and was most pronounced on 2,4-D levels above 50 mg/l. Coconut water had a significant inhibitory effect on callusing of older tissue, but it enhanced callusing in younger tissue. It had no effect on organogenesis, but reduced discoloration-significantly.

A histological section shows a shoot-tip with two leaf primordia, in which the apical meristem is completely enclosed. The tissue of 4-month-old calli consisted of small, evenly-shaped cells with well-stained nuclei. Occasionally, distinct groupings of cells were detected, suggesting the possibility of pro-embryonic formations, but distinct bipolarity was not recognizable in these cell clumps.

INTRODUCTION

Activated charcoal is best known for its adsorption of metabolites which are detrimental to explants in tissue culture (Fridborg and Eriksson, 1975; Fridborg et al., 1978; Wang and Huang, 1976; Weatherhead et al., 1978 and 1979). It has been shown that the addition of activated charcoal to the culture medium improves growth (Wang and Huang, 1976), and enhances embryogenesis and organogenesis (Drew, 1979; Salazar, 1986). A few reports indicate that activated charcoal is needed in the medium for enhanced callus production as well (Sharma, 1984; Tisserat, 1979). The presence of activated charcoal has also reduced browning in palm tissues to some degree (Wang and Huang, 1976), which may be attributed to its ability to adsorb phenolic compounds (Weatherhead et al., 1979). Activated charcoal adsorbs chemicals in a selective fashion: it removes thiamine HCl and nicotinic acid from the medium, for example, but not sucrose or inositol (Weatherhead et al., 1978). NAA can be adsorbed completely in concentrations up to 300 mg/l, and cytokinins are affected, too (Weatherhead et al., 1979). These latter observations need to be kept in mind when adding growth regulators to tissue culture media containing activated charcoal, because the growth regulators may become unavailable.

Coconut milk has been reported to enhance culture growth (Erdelsky and Cholvadtova, 1970) and promote the germination of 'Makapuno' coconut embryosgrown <u>in vitro</u>, which do not germinate under natural conditions (Balaga and de Guzman, 1971). It has been reported, however, that the stimulatory effect of coconut milk is not always consistent (Rabéchault et al., 1972), and that it can even have adverse effects on tissue cultures in certain instances (Cutter and

Wilson, 1954). Auxin and gibberellin-like substances have been identified in coconut milk (van Staden and Drewes, 1975, and Dix and van Staden, 1982), but when these were isolated and added separately to the culture medium, they failed to induce the response observed when unextracted coconut milk was added. This suggests the presence of unidentified substances in coconut milk which promote growth (Dix and van Staden, 1982). Although coconut milk contains both auxins and cytokinins, its cytokinin-like activity seems to dominate, since it may be substituted for kinetin in supporting the growth of tissues cultured in vitro (van Staden and Drewes, 1975). It is not clear whether autoclaving has a detrimental effect on the activity of coconut milk. One report indicates that autoclaved coconut milk has a higher degree of activity in liquid, aerated cultures than in solid cultures, without suffering any detrimental effects during the autoclaving process per se (Rabéchault et al., 1972). However, a report published one year later by the same authors (Rabéchault at al., 1973) describes the inactivation of autoclaved coconut milk as a germination promoter for rehydrated coconut seeds. In this later study, filtered, non-autoclaved coconut milk proved to be much more active. Cutter and Wilson (1954) indicated that some favorable factor in coconut milk from young coconuts is not heat stable.

The developmental stage of cells in a given explant plays an important role in the responsiveness of the explant. "In general, younger tissues, such as terminal or axillary shoot-tips...will regenerate better than older, more mature tissues..." (Hartmann and Kester, 1983). Cuttings taken from juvenile hardwoods are usually easier to root than if they are taken from mature plants (Hartmann and Kester, 1983), and it is therefore conceivable that juvenility has a positive effect upon in vitro propagation.

It was shown in at least one report on palm tissue culture that higher callusing and embryogenesis rates occurred in explants from juvenile plants, while lower rates were obtained on explants of mature palms (Hanower and Pannetier, 1982, in Paranjothy, 1986). Pinedo Panduro (1987) mentions the possibility that juvenile tissue enhances embryogenic responses. Along with a study of the effect of various 2,4-D levels, coconut water, and activated charcoal on pejibaye shoot tip cultures, this experiment tests whether a difference exists between 1½-year-old seedlings and 4-month-old seedlings in terms of their in vitro responses.

MATERIALS AND METHODS

Experiment 1: Tissue Culture of Shoot Tips

A completely randomized design was set up in which 162 shoot-tips were distributed over 18 treatments, with nine replications per treatment. The pH of the media preparations was adjusted to 5.8, after which the media were autoclaved for 15 minutes at 1.1 kg/cm^2 and 121°C. Subsequently, 15 ml-aliquots of the media were dispensed into disposable 6 cm petri dishes. All treatments consisted of an MS (1962) medium, supplemented by the ingredients specified in Table IV-1.

Seedlings were imported from Costa Rica when they had just emerged from the seeds, and were grown in individual pots in a greenhouse at 27°C. No artificial light was used. The seedlings were watered every other day and fertilized every other week with a soluble greenhouse fertilizer containing 200 ppm N. Iron chelate was added to the fertilizer if chlorosis symptoms appeared.

Explants were obtained by cutting off the roots, foliage, and part of the stem of the palm seedlings, until 9-cm stem segments were left which included the broad base of the seedling. These segments were rinsed several times to remove the soil. The outermost tissue layer (the base of a leaf sheath) of each stem segment was peeled off, and the stem segments were subsequently surface sterilized by immersing them in 70 % ethyl alcohol for one minute. This step was followed by a transfer of the stem segments into a 30 % (v/v) chlorine bleach solution containing 2 drops of the surfactant "Tween 80", in which they were shaken continuously for 15 minutes.

Upon completion of this step the segments were rinsed 3-4 times with sterile deionized water under aseptic conditions. Two more tissue layers or leaf sheath bases were then removed from the segments, which left stem sections formed by the inner two or three layers of leaf-sheath tissue enclosing the leaf primordia, with the broad stem base still attached at the proximal end, where the roots had been attached. To obtain the shoot tips, stem segments were held by hand at their distal end, where the foliage was originally attached, and thin slices were cut from the broad stem base. Contamination was avoided by holding the distal end outside the petri dish, while resting the proximal end inside the petri dish in which the dissection was carried out. After a few slices had been removed from the broad stem base, several concentric "rings" became visible in the stem tissue. This indicated that the cuts had reached an area very close to the shoot tip.

At this point a circle was cut into the stem tissue following the contour of the innermost "ring" visible in the center of the cut stem surface. By squeezing the base of the stem section gently between two fingers, the shoot tip, with a thin layer (1-2 mm) of stem tissue still attached at its base, was ejected from the stem section without suffering any visible damage (Fig. IV-1). The shoot tips, which were between 2 and 3 mm in length, were placed vertically on the medium, analogous to their natural orientation in the seedling. The cultures were placed in a dark growth chamber at 24±1°C, from which they were removed for observation once a week. The data presented in this paper were collected at the end of four months. Responses were grouped into three major categories: callusing, discoloration, and organogenesis.

Because there were clearly distinguishable differences in the degree to which shoot tips formed calli, the calli were assigned scores ranging from 0 to 3. By the terms of this subjective scale, 0 was assigned whenever no callus was visible; 1 when only small areas of callus were visible; 2 if callusing occurred over at least 50 % of the explant and was of intermediate size (about 3 mm in diameter); and 3 if the shoot tip had callused completely and the callus clump appeared to be growing actively (3-7 mm in diameter). Organogenesis in the context of this experiment implies the development of structures that are clearly not calli, without making a distinction between those that arise adventitiously and those that arise from pre-existing organs. Explants were categorized as discolored whenever there was a complete lack of growth or change in the explant from its original form, accompanied by a fading of the original white-cream color to the point of acquiring an almost translucent quality (Fig. IV-2). The results contained several missing values due to contamination, and therefore Type III Sums of Squares were used in the analyses of variance.

Experiment 2: Histological Analysis

A pejibaye shoot-tip was obtained following the method outlined above. It was fixed immediately, then dehydrated and embedded in paraffin, after Johansen (1940). Tissue slices were cut on a microtome at settings of 10 and 15 µm, mounted on microscope slides and stained as indicated, using safranin and chlorazol black E as stain and counterstain respectively (Johansen, 1940).

RESULTS

Experiment 1: Tissue Culture of Shoot Tips

Responses ranged widely, reflecting the wide variety of media compositions used. Callusing in shoot tips is quite different from callusing in leaf-sheath sections or subapical stem tissue (see PART II and PART III). It develops throughout the entire explant (not just the upper cut surface), the consistency of calli in shoot tips is more globular, and the color is creamy to light brown (Figure IV-3). All four variables, i.e., age, activated charcoal, coconut water, and 2,4-D levels, had significant effects on callus production (Table IV-2). Both in terms of overall callusing percentage and scoring, callusing was highest on 25 and 50 mg/l 2,4-D, if no activated charcoal was present (Tables IV-3 through IV-5, Figs. IV-4 and IV-5). The highest callusing scores, however, did not necessarily parallel the highest callusing percentages (Table IV-6).

Activated charcoal had a strongly inhibiting effect upon callus formation, as did its interaction with most of the variables involved (Table IV-2, Figs. IV-4 through IV-6). In the presence of activated charcoal, callusing was only observed on 2,4-D concentrations above 50 mg/l (Fig. IV-5). Even though the presence of coconut water was significant at the 1 % level (Table IV-2), the nature of its activity was not unidirectional, since it had both positive and negative effects, depending on its interaction with other variables (Tables IV-3 through IV-5, Figs. IV-7 through IV-9). As an isolated variable, coconut water tended to inhibit callusing, especially at 2,4-D levels between 0 and 50 mg/l (Table IV-3, Fig. IV-8), as it did in combination with activated charcoal (Fig IV-7).

At 2,4-D levels from 75-100 mg/l, which by themselves inhibited callusing (Table IV-3), coconut water enhanced callus production (Fig. IV-7). The activity of coconut water was expressed most distinctly in its interaction with age, where it discouraged callusing in older tissue, but encouraged callusing in younger tissue (Tables IV-4 and IV-5, Fig. IV-9).

In general, callusing was most pronounced in shoot-tips from 4-month-old seedlings (Table IV-3). At individual 2,4-D concentrations, young tissue callused best at 25 and 50 mg/l (Figs. IV-9 and IV-10), whereas callusing declined at concentrations above 50 mg/l. This pattern was reversed in combination with activated charcoal, where younger tissue callused better at higher concentrations of 2,4-D than older tissue (Fig. IV-6).

Organogenesis took the form of: roots; elongation of simple or multiple shoots together with leaf development; the development of both types of organs on the same explant; or a broadening ("fattening") of the shoot tip base. In some instances, organogenesis consisted of a non-definable but organized mass of convoluted tissue with no clear organ development (Fig. IV-11). The only variable that had a strong influence on organogenesis was the presence of activated charcoal, with the interaction between activated charcoal and 2,4-D levels also playing a significant role (Table IV-7). Treatments containing activated charcoal had the highest organogenesis rates both at 0 and 75-100 mg/l 2,4-D (Tables IV-3 and IV-4, and Fig. IV-12). The percentage of cultures exhibiting organogenesis was always higher with activated charcoal in the medium, than without (Fig. IV-13). Without activated charcoal in the medium, organogenesis was encouraged only at the lower concentrations of 2,4-D (Table IV-3, and Figs. IV-12 and IV-13).

In summary, organogenesis rates were highest on 100 mg/l 2,4-D and activated charcoal, with the formation of mostly undefined growth; on 75 mg/l 2,4-D, with activated charcoal and coconut water, favoring root production; on 25 mg/l 2,4-D, with activated charcoal, producing both roots and shoots; and on 0 mg/l 2,4-D, with activated charcoal and coconut water, leading to shoot elongation and leaflet development (Fig. IV-13). If roots and shoots were present on the same explant, one of the two types of organs was usually dominant. Coconut water did not have any significant effect on organogenesis (Table IV-7).

The 2,4-D concentration was significant in determining the presence or absence of discoloration (Table IV-8). Discoloration occurred in 13.6 % of the explants, most markedly so on media with 2,4-D concentrations above 50 mg/l without coconut water or activated charcoal (Tables IV-3 through IV-5). Both activated charcoal and coconut water had a significant inhibitory effect on discoloration, as did their interaction (Table IV-8). Although the effect of both substances was highly significant, they each inhibited discoloration to different degrees, with activated charcoal being the most effective of the two. Fig. IV-14 shows that activated charcoal completely inhibits discoloration, whereas coconut water only reduces it in most cases, as reflected in Fig. IV-15. There was no discoloration whenever activated charcoal and coconut water were added together (Fig. IV-16).

Contamination was 10.5 % (9.9 % bacterial contamination, 0.6 % fungal contamination), most of it concentrated in explants taken from 4-month-old seedlings. As a result, bacterial contamination was significant at the 5 % level for 4-month-old seedlings. Browning was observed in only a handful of cultures, and if so, it was barely detectable.

Experiment 2: Histological Analysis

The longitudinal section of the shoot-tip (Fig. IV-17) shows that the apical meristem is contained well within the shoot-tip that was extracted by the method described above. The apical meristem is enclosed within two leaf primordia, with the outermost leaf primordium constituting the shoot-tip extracted from the base of the pejibaye seedling. It is difficult to determine from Fig. IV-17 whether any axillary meristems are present in the apical meristem. Shoot-tip calli consisted of small, evenly-shaped cells (Fig. IV-18), much unlike the calli of leaf-sheath explants (see PART II), which were very large and uneven. Occasionally, distinct groupings of cells were detected, suggesting the possibility of pro-embryonic formations. No bipolarity was recognized in these cell clumps, however.

DISCUSSION AND CONCLUSIONS

As expected, shoot tips produced good calli for medium combinations favoring such a response, since shoot tips are highly meristematic tissues, as the intense staining of the shoot-tip section confirms (Figure IV-17). Shoot tips have been used successfully by all previous researchers of pejibaye tissue culture (Arias, 1985; Arias and Huete, 1983; Salazar, 1986; Pinedo Panduro, 1987; Valverde and Arias, 1986a and 1986b; Valverde et al., 1987). The strongly inhibiting effect that activated charcoal at 2.5 g/l has upon callus initiation in pejibaye shoot-tips can be explained by the finding of Weatherhead et al. (1978) that activated charcoal can adsorb auxins quite effectively, thus rendering them ineffective for callus initiation. The same theory could also be used to explain why activated charcoal favored organogenesis at high 2,4-D concentrations: if 2,4-D was adsorbed strongly, its effectiveness as a callus-inducing agent would be reduced, and organ development rather than dedifferentiation would ensue.

Coconut water does not play an important role in organogenesis and should therefore not be used for that purpose in pejibaye tissue culture. The fact that it reduces callusing in older tissue reinforces van Staden's and Drewes' statement (1974) that it acts more like a cytokinin than like an auxin. Why coconut water and explants from very young tissues are synergistic is not clear, but it indicates that plant age differences play a role in pejibaye tissue culture. Even though both the 4-month-old and the 1½-year-old palms can be considered juvenile, the 1-year age difference between them does influence <u>in vitro</u> responses. This assertion is corroborated by the enhanced callusing rates in the shoot tips of 4-month-old seedlings.

The combined presence of callusing and organogenesis on a single treatment, and even within a single culture, indicates that responses are frequently not clear-cut. This appeared to be especially the case whenever the organogenic portion of the response fit into the "undefined" category. It is possible that such undefined growth is due to a strong tendency in both directions, i.e., towards callusing and organogenesis simultaneously, and that the explant in question is prevented from taking a clear course. It seems plausible that in those cases where roots were better developed than shoots on the same explant, shoot formation was suppressed, and vice versa. This phenomenon may be attributed to the medium composition, in which case the formulation of the culture medium would need to be altered in order to encourage the growth of the lesser developed organ, and thus obtain complete plantlets.

The histological analysis suggests that the procedure for obtaining shoot tips followed in this experiment is safe and does not damage the apical meristem enclosed in the shoot-tip. Pinedo Panduro (1987) and Salazar (1986) obtained shoot-tips by cutting out a small cube of stem tissue from pejibaye offshoots or seedlings which included the shoot-tip. These cubes became the explants and had to be sterilized once they were excised, before being plated onto the culture medium. Using the procedure followed here, only the stem segments obtained from the seedlings have to be surface sterilized. Any explants dissected from the stem segment, be they shoot tips, leaf-sheath slices, or subapical stem slices, can be plated out directly, without subjecting them to the stress of any further sterilization procedures. It would be worthwhile to attempt using this dissection method with older seedlings or offshoots, i.e., plants with thicker stems.

Several of the organogenic responses obtained here were similar to those described by Pinedo Panduro (1987). He obtained up to 14 shoots from one shoot-tip cultured in vitro on a modified MS medium containing 10 mg/l NAA, and indicated that they may have arisen either adventitiously or from pre-existing primordia. The histological section of the shoot-tip shows only three leaf primordia, with no axillary meristems visible at a 20.5x magnification, which suggests that if numerous shoots are obtained from a single shoot tip, most of them arise adventitiously, since no further growing points were detected. Finer histological methods are needed to ascertain the presence or absence of axillary shoot promeristems, and therefore to ascertain the hypothesis that multiple shoots originate de novo.

REFERENCES CITED

Arias, Oscar. 1985. Propagación vegetativa por cultivos de tejidos del pejibaye (Bactris gasipaes H.B.K.). ASBANA 24(9):24-27.

Arias M., Oscar, and Fernando Huete V. 1983. Propagación vegetativa in vitro de pejibaye (Bactris gasipaes H.B.K.). Turrialba 33(2):103-108.

Balaga, H. Y., and E. V. de Guzman. 1971. The growth and development of coconut "Makapuno" embryos in vitro. II. Increased root incidence and growth in response to media composition and to sequential culture from liquid to solid medium. Philipp. Agric. 53(10):551-565.

Cutter, V. M., Jr., and Katherine S. Wilson. 1954. Effect of coconut endosperm and other growth stimulants upon the development in vitro of embryos of Cocos nucifera. Bot. Gaz. 115:234-240.

Dix, L., and J. van Staden. 1982. Auxin and gibberellin-like substances in coconut milk and malt extract. Plant Cell Tiss. Org. Cult. 1(4):239-246.

Drew, R. L. K. 1979. Effect of activated charcoal on embryogenesis and regeneration of plantlets from suspinyension cultures of carrot (Daucus carota L.). Ann. Bot. 44:387-389.

Erdelsky, Karol, and Zdena Cholvadtova. 1970. Wirkung von Kokosmoothilch und Hefeextrakt auf das Wachstum von Gewebekulturen der Tabakpflanze Nicotiana tabacum L. (The effect of coconut milk and yeast extract on the growth of tissue culture of tobacco Nicotiana tabacum L. Acta Fac. Rerum. Nat. Comenianae Physiol. Plant. 1:99-116. (English summary).

Fridborg, Gunnar, and Tage Eriksson. 1975. Effects of activated charcoal on growth and morphogenesis in cell cultures. Physiol. Plant. 34:306-308.

Fridborg, Gunnar, Marianne Pedersén, Lars-Erik Landström, and Tage Eriksson. 1978. The effect of activated charcoal on tissue cultures: Adsorption of metabolites inhibiting morphogenesis. Physiol. Plant. 43(2):104-106.

Halperin, Walter. 1986. Attainment and retention of morphogenetic capacity in vitro. Pages 3-47 in Vasil, Indra K., ed. Cell culture and somatic cell genetics of plants. Vol. 3. Academic Press, Inc., New York.

Hartmann, Hudson T., and Dale E. Kester. 1983. Plant propagation. 4th edition. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. 727 pp.

Johansen, Donald Alexander. 1940. Plant microtechnique. McGraw-Hill, New York. 523 pp.

Murashige, Toshio and Folke Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15(3):473-497.

Paranjothy, K. 1986. Recent developments in cell and tissue culture of oil bearing palms. PORIM (Palm Oil Res. Inst., Malaysia) Occasional Paper No.19. 12 pp.

Pinedo Panduro, Mario Herman. 1987. Organogénesis directa en ápices caulinares de pejibaye (Bactris gasipaes H.B.K.). Master's Thesis. Centro Agronómico Tropical de Investigación y Enseñanza, Turrialba, Costa Rica. 110 pp.

Rabéchault, H., J. Ahée, and G. Guénin. 1972. Recherches sur la culture in vitro des embryons de palmier à huile (Elaeis guineensis Jacq. var. Dura Becc.). VIII. Action du lait de coco autoclavé en présence ou non de gélose et de lumière, et en raison de l'âge des graines (The in vitro culture of oil palm embryos, Elaeis guineensis var. dura. VIII. The action of autoclaved coconut milk in the presence or absence of agar and light and in relation to seed age). Oléagineux 27(5):249-254. (English summary).

Rabéchault, H., G. Guénin, and J. Ahée. 1973. Recherches sur la culture in vitro des embryons de palmier à huile (Elaeis guineensis Jacq. var. Dura Becc.). IX. Activation de la sensibilité au lait de coco par une réhydratation des graines (The in vitro culture of oil palm embryos, Elaeis guineensis var. dura. IX. Activation of the response to cocnut milk by rehydration of seeds). Oléagineux 28(7):333-336. (English summary).

Salazar, Sebastián S. 1986. The effect of 2,4-D, light, and activated charcoal on offshoot-tip culture of the pejibaye palm (Bactris gasipaes H.B.K.). Master's Thesis. University of Birmingham, Birmingham, England. 70 pp.

Valverde, Roberto, and Oscar Arias. 1986a. Efecto de la luz sobre la morfogénesis de tejidos de pejibaye cultivados in vitro. Pages 356-358 in VII Congreso Agronómico Nacional, XXXIII Congreso de Horticultura (A.S.H.S.-Región Tropical), vol. 1. Colegio de Ingenieros Agrónomos de Costa Rica and Sociedad Americana de Ciencias Hortícolas Región Tropical (A.S.H.S.), San José, Costa Rica. (Abstr.)

Valverde, Roberto, and Oscar Arias. 1986b. Efecto del picloram sobre la morfogénesis del pejibaye (Bactris gasipaes H.B.K.) in vitro. Pages 358-359 in VII Congreso Agronómico Nacional, XXXIII Congreso de Horticultura (A.S.H.S.-Región Tropical). Vol 1. Colegio de Ingenieros Agrónomos de Costa Rica and Sociedad Americana de Ciencias Hortícolas Región Tropical (A.S.H.S.), San José, Costa Rica. (Abstr.)

Valverde, Roberto, Oscar Arias, and Trevor A. Thorpe. 1987. Picloram-induced somatic embryogenesis in pejibaye palm (Bactris gasipaes H.B.K.). Plant Cell, Tissue and Organ Culture 10:149-156.

van Staden, J., and S. E. Drewes. 1974. Identification of cell division inducing compounds from coconut milk. Physiol. Plant. 32(4):347-352.

van Staden, J., and S. E. Drewes. 1975. Identification of zeatin and zeatin riboside in coconut milk. Physiol. Plant. 34:106-109.

Wang, P.-J., and L.-C. Huang. 1976. Beneficial effects of activated charcoal on plant tissue and organ cultures. In Vitro 12:260-262.

Weatherhead, M. A., J. Burdon, and G. G. Henshaw. 1978. Some effects of activated charcoal as an additive to plant tissue culture media. Z. Pflanzenphysiol. 89(2):141-147.

Weatherhead, M. A., J. Burdon, and G. G. Henshaw. 1979. Effects of activated charcoal as an additive plant tissue culture media: Part 2. Z. Pflanzenphysiol. 94(5):399-405.

Table IV-1. Composition of the treatments utilized in Experiment 1. ("AC" stands for activated charcoal, "COC" for deproteinized coconut water, and "Y" for the presence of activated charcoal or coconut water in the treatment)

TREATMENT No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
2,4-D (mg/l)	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100	0	0
BA (mg/l)	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0
COC (100ml/l)	--	--	--	--	--	--	--	--	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
AC (2.5 g/l)	Y	Y	Y	Y	--	--	--	--	Y	Y	Y	Y	--	--	--	--	--	Y

Table IV-2. Analysis of variance for callusing of pejibaye shoot tips. ("AGE" differentiates between 1½-year-old seedlings and 4-month-old seedlings, "AC" = activated charcoal, and "COC" = deproteinized coconut water)

SOURCE	df	TYPE III SS	f VALUE
AGE	1	2.11	10.29**
AC	1	9.80	47.78***
AGE*AC	1	0.63	3.07ns
COC	1	1.45	7.09**
AGE*COC	1	7.34	35.81***
AC*COC	1	0.39	1.93ns
AGE*AC*COC	1	6.06	29.57***
2,4-D	4	11.38	13.88***
AGE*2,4-D	3	2.02	3.28*
AC*2,4-D	4	16.50	20.12***
AGE*AC*2,4-D	3	1.44	2.34ns
COC*2,4-D	3	5.55	9.03***
AGE*COC*2,4-D	3	1.91	3.11ns
AC*COC*2,4-D	3	3.49	5.67***
AGE*AC*COC*2,4-D	3	2.20	3.58*
ERROR	112	22.97	
CORRECTED TOTAL	145	84.75	

*Significant at the 5 % level.
**Significant at the 1 % level.
***Significant at the 0.1 % level.
ns=Non-significant.

Table IV-3. Callusing, organogenesis, and discoloration means for the effects of pejibaye seedling age, activated charcoal, coconut water, and 2,4-D. ("Old" refers to 1½-year-old seedlings, "Young" to 4-month-old seedlings. Callus scores were assigned as follows for each individual culture: 0 = no callusing; 1 = small areas of callus visible on the explant; 2 = at least 50 % of the explant had callused; and 3 = complete callusing exhibiting vigorous growth. Means for callusing are all lower than 1, because they are the means for the entire experiment, and not just for individual treatments)

MEANS:	CALLUSING (Score)	ORGANOGENESIS (% Cultures)	DISCOLORATION (% Cultures)
VARIABLES:			
AGE			
Old	0.37	28.1	18.0
Young	0.42	40.3	10.5
ACTIVATED CHARCOAL (2.5 g/l)			
Yes	0.12	51.3	0.0
No	0.67	13.9	30.5
COCONUT WATER (100 ml/l)			
Yes	0.33	34.6	7.7
No	0.45	30.9	23.5
2,4-D (mg/l)			
0	0.00	50.0	0.0
25	0.53	35.3	5.9
50	0.68	19.3	9.7
75	0.26	29.0	29.0
100	0.31	37.5	25.0

Table IV-4. Means for first-order interactions of the culture media variables utilized in the in vitro culture of pejibaye shoot tips. ("Old" refers to 1½-year-old seedlings, "Young" to 4-month-old seedlings. Callus scores were assigned as follows for each individual culture: 0 = no callusing; 1 = small areas of callus visible on the explant; 2 = at least 50 % of the explant had callused; and 3 = complete callusing exhibiting vigorous growth. Means for callusing are all lower than 1, because they are the means for the entire experiment, and not just for individual treatments)

MEANS:		CALLUSING (Score)	ORGANOGENESIS (% Cultures)	DISCOLORATION (% Cultures)
VARIABLES:				
AGE	ACTIVATED CHARCOAL (2.5 g/l)			
Old	Yes	0.11	48.9	0.0
Old	No	0.64	6.8	36.4
Young	Yes	0.14	55.2	0.0
Young	No	0.71	25.0	21.4
AGE	COCONUT WATER (100 ml/l)			
Old	Yes	0.21	26.2	14.3
Old	No	0.51	29.8	21.3
Young	Yes	0.47	44.4	0.0
Young	No	0.33	33.3	28.6
ACTIVATED CHARCOAL (2.5 g/l)	COCONUT WATER (100 ml/l)			
Yes	Yes	0.13	51.3	0.0
Yes	No	0.11	51.4	0.0
No	Yes	0.54	17.9	15.4
No	No	0.82	9.1	48.5

Table IV-5. Means of the second-order interactions among age, activated charcoal, and coconut water in pejibaye shoot-tip cultures. ("Old" refers to 1½-year-old seedlings, "Young" to 4-month-old seedlings. Callus scores were assigned as follows for each individual culture: 0 = no callusing; 1 = small areas of callus visible on the explant; 2 = at least 50 % of the explant had callused; and 3 = complete callusing exhibiting vigorous growth. Means for callusing are all lower than 1, because they are the means for the entire experiment, and not just for individual treatments. Differences in organogenesis were non-significant and are therefore omitted)

MEANS:			CALLUSING (Score)	DISCOLORATION (% Cultures)
VARIABLES				
AGE	ACTIVATED CHARCOAL (2.5 g/l)	COCONUT WATER (100 ml/l)		
Old	Yes	Yes	0.14	0.0
Old	Yes	No	0.08	0.0
Old	No	Yes	0.28	28.6
Old	No	No	0.96	43.5
Young	Yes	Yes	0.11	0.0
Young	Yes	No	0.18	0.0
Young	No	Yes	0.83	0.0
Young	No	No	0.50	60.0

Table IV-6. A comparison between the percent of pejibaye shoot-tip cultures forming calli on given treatments and their corresponding average callus score. (Callus scores were assigned as follows: 0 = no callusing; 1 = small areas of callus visible on explant; 2 = at least 50 % of the explant had callused; and 3 = complete callusing exhibiting vigorous growth. AC stands for 2.5 g/l activated charcoal, COC for 100 ml/l deproteinized coconut water)

TREATMENT	25 mg/l 2,4-D (No AC, no COC)	50 mg/l 2,4-D (No AC, no COC)	50 mg/l 2,4-D (No AC, yes COC)
CALLUSING (%)	89	67	56
SCORE	1.7	2.0	1.8

Table IV-7. Analysis of variance for organogenesis in pejibaye shoot-tip cultures. ("AGE" differentiates between 1½-year-old seedlings and 4-month-old seedlings, AC stands for 2.5 g/l activated charcoal, COC for 100 ml/l deproteinized coconut water)

SOURCE	df	TYPE III SS	f VALUE
AGE	1	0.14	0.78ns
AC	1	4.73	26.99***
AGE*AC	1	0.00	0.01ns
COC	1	0.06	0.35ns
AGE*COC	1	0.14	0.78ns
AC*COC	1	0.00	0.01ns
AGE*AC*COC	1	0.00	0.01ns
2,4-D	4	0.95	1.35ns
AGE*2,4-D	3	0.20	0.39ns
AC*2,4-D	4	1.92	2.74*
AGE*AC*2,4-D	3	0.04	0.07ns
COC*2,4-D	3	0.51	0.98ns
AGE*COC*2,4-D	3	0.47	0.89ns
AC*COC*2,4-D	3	0.79	1.51ns
AGE*AC*COC*2,4-D	3	0.98	1.87ns
ERROR	112	19.64	
CORRECTED TOTAL	145	32.22	

*Significant at the 5 % level.
***Significant at the 0.1 % level.
ns=Non-significant.

Table IV-8. Analysis of variance for discoloration of pejibaye shoot tips cultured in vitro. ("AGE" differentiates between 1½-year-old seedlings and 4-month-old seedlings, AC stands for 2.5 g/l activated charcoal, COC for 100 ml/l deproteinized coconut water)

SOURCE	df	TYPE III SS	f VALUE
AGE	1	0.00	0.05ns
AC	1	3.51	71.57***
AGE*AC	1	0.00	0.05ns
COC	1	0.96	19.51***
AGE*COC	1	0.45	9.13**
AC*COC	1	0.96	19.51***
AGE*AC*COC	1	0.45	9.13**
2,4-D	4	0.86	4.41**
AGE*2,4-D	3	0.10	0.71ns
AC*2,4-D	4	0.86	4.41**
AGE*AC*2,4-D	3	0.10	0.71ns
COC*2,4-D	3	1.18	8.00***
AGE*COC*2,4-D	3	0.24	1.65ns
AC*COC*2,4-D	3	1.18	8.00***
AGE*AC*COC*2,4-D	3	0.24	1.65ns
ERROR	112	5.50	
CORRECTED TOTAL	145	18.68	

**Significant at the 1 % level.
***Significant at the 0.1 % level.
ns=Non-significant.

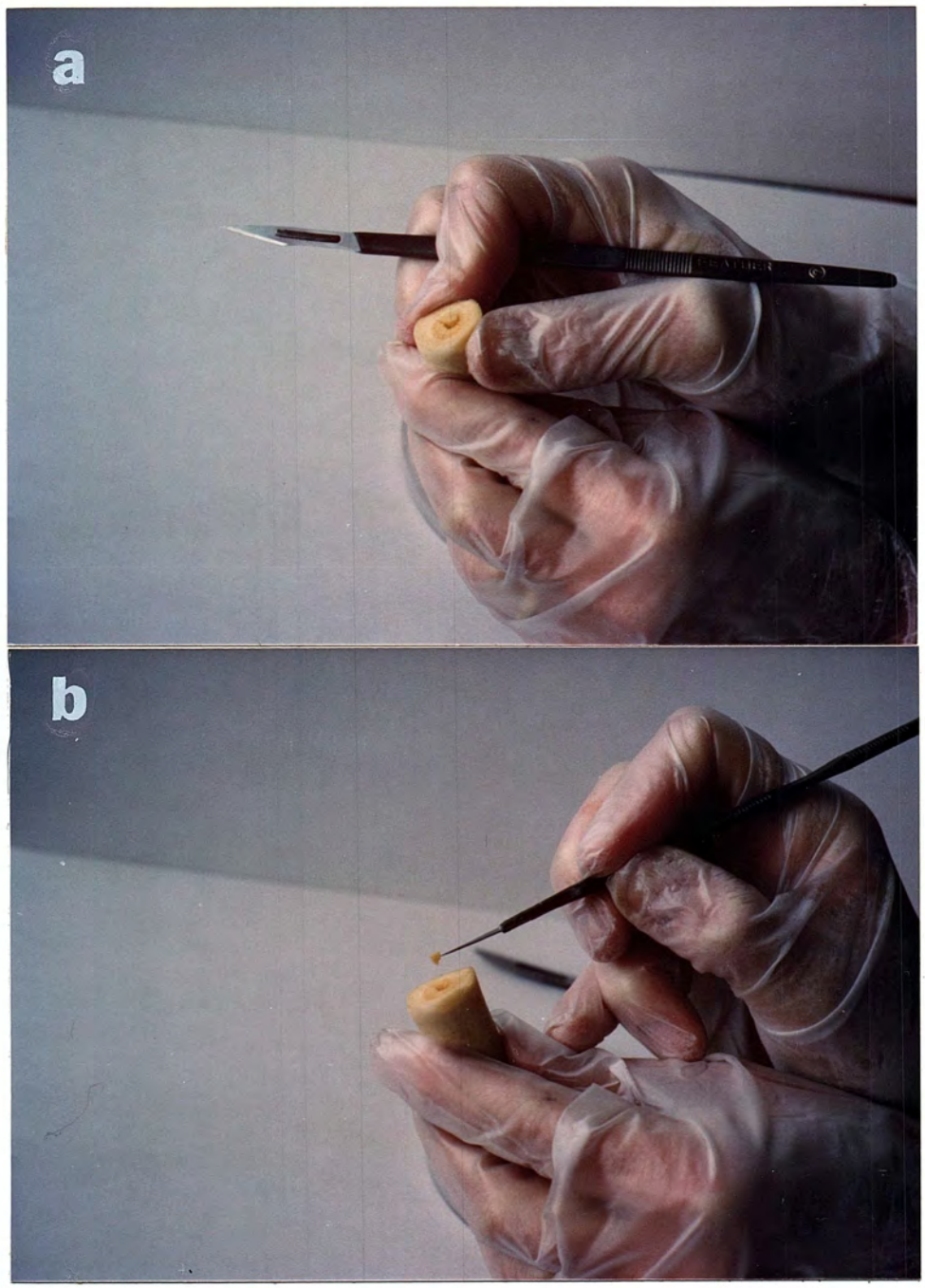


Figure IV-1. Extraction of a pejibaye shoot tip. (a) Removal of a shoot tip from a pejibaye seedling by squeezing the broad stem base. (b) The shoot tip is subsequently lifted from the stem with a pointed scalpel

Figure IV-2. Discoloration of a pejibaye shoot tip. The paling of the tissue makes it almost indistinguishable from its background. Compare with creamy color of fresh shoot tip in Fig. IV-1

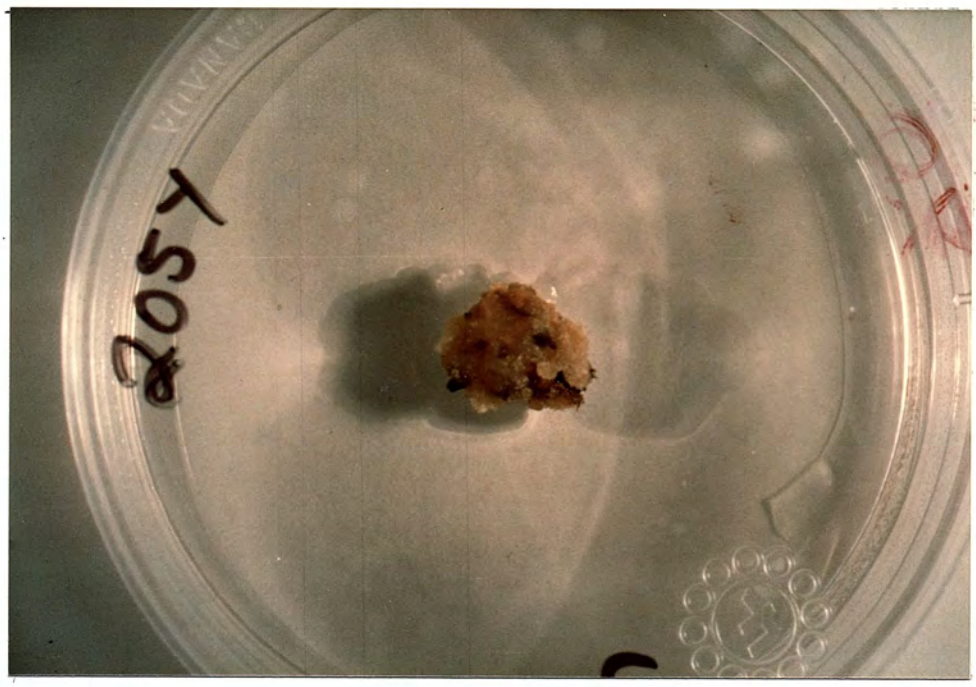
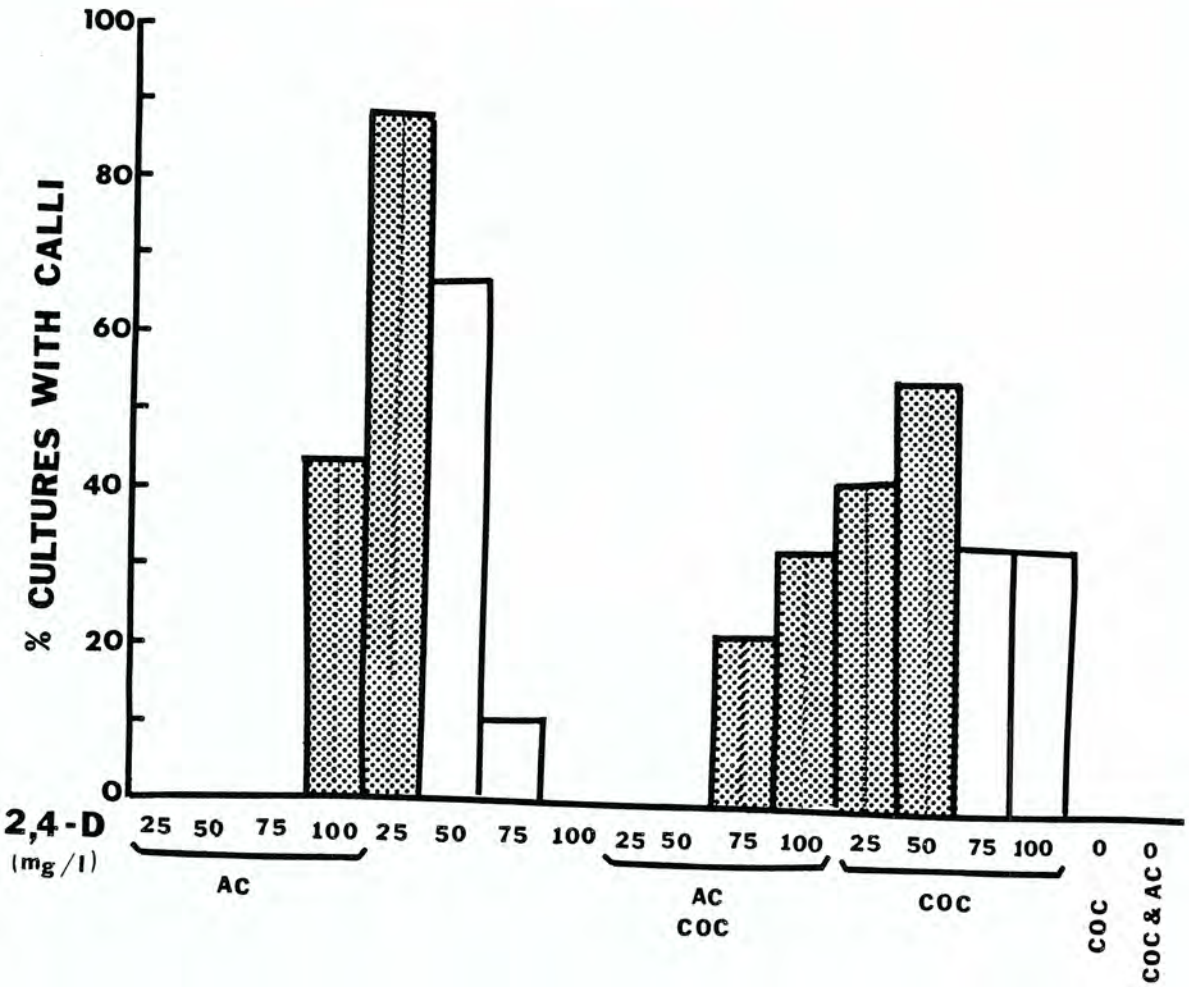


Figure IV-3. Callus formation on a pejibaye shoot-tip cultured on an MS (1962) medium with 25 mg/l 2,4-D and 5 mg/l BA



= cultures with callus formation only

= cultures exhibiting both callusing and organogenesis

AC = 2.5 g/l activated charcoal added
COC = 100 ml/l coconut water added

Figure IV-4. Callusing rates in pejibaye shoot tips cultured on a variety of media containing five levels of 2,4-D (0, 25, 50, 75, and 100 mg/l) and combinations of coconut water and activated charcoal. All media contained 5 mg/l BA, except when 2,4-D levels were 0, in which case no BA was added either

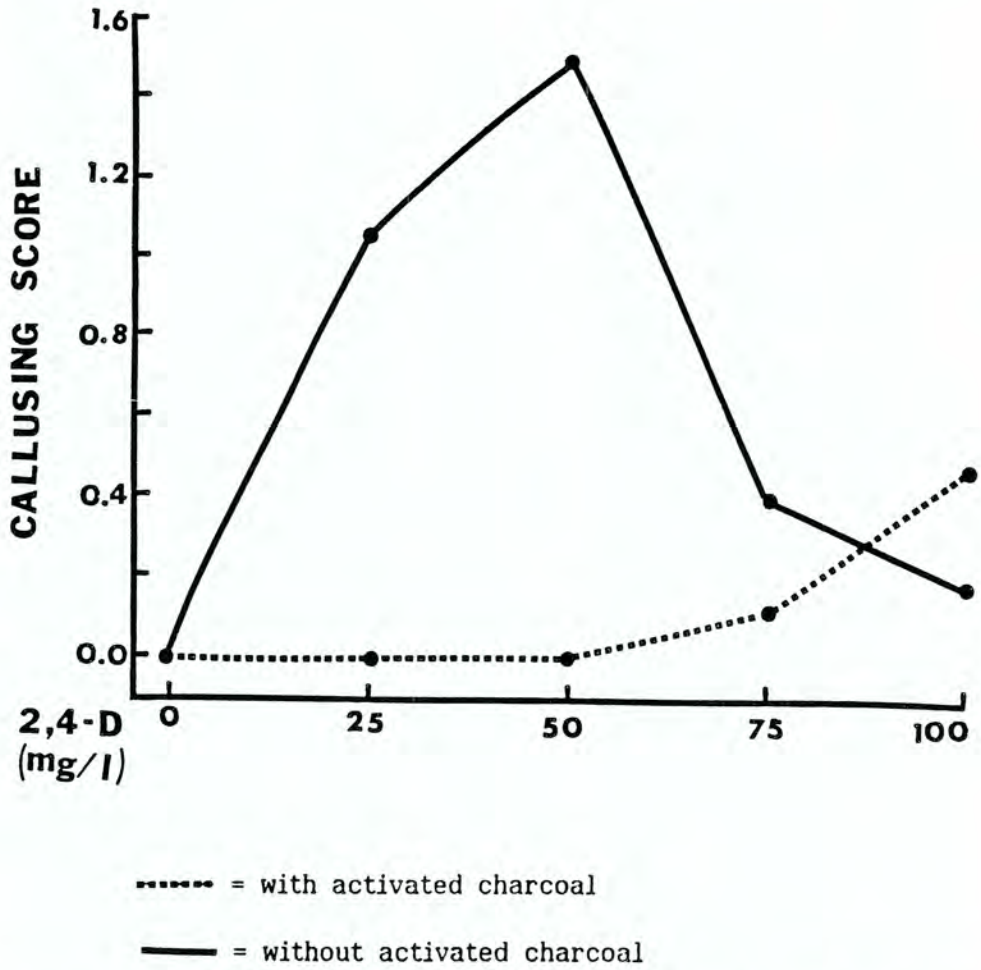


Figure IV-5. Mean callusing scores of pejibaye shoot tips for the interaction between activated charcoal and 2,4-D. Activated charcoal was added at 2.5 g/l. (Callus scores were assigned as follows: 0 = no callusing; 1 = small areas of callus visible on explant; 2 = at least 50 % of the explant had callused; and 3 = complete callusing exhibiting vigorous growth. Means for callusing are all lower than 1, because they are the means for the entire experiment, and not just for individual treatments.)

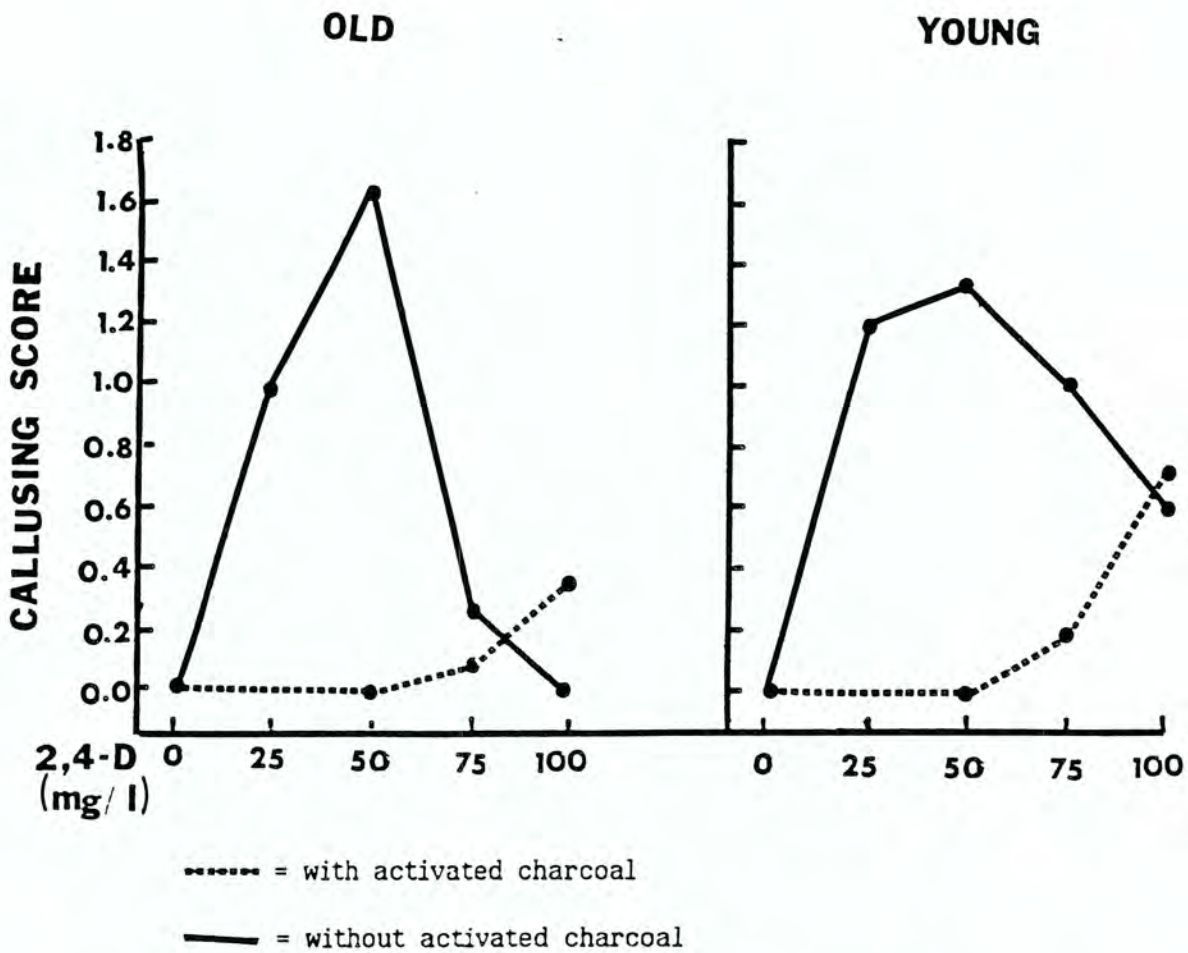


Figure IV-6. Mean callusing scores of pejibaye shoot tips for the interaction among age, activated charcoal, and 2,4-D. Activated charcoal was added at 2.5 g/l. ("Young" refers to 4-month-old seedlings, "Old" to 1½-year-old seedlings. Callus scores were assigned as follows: 0 = no callusing; 1 = small areas of callus visible on the explant; 2 = at least 50 % of the explant had callused; and 3 = complete callusing exhibiting vigorous growth. Means for callusing are all lower than 1, because they are the means for the entire experiment, and not just for individual treatments.)

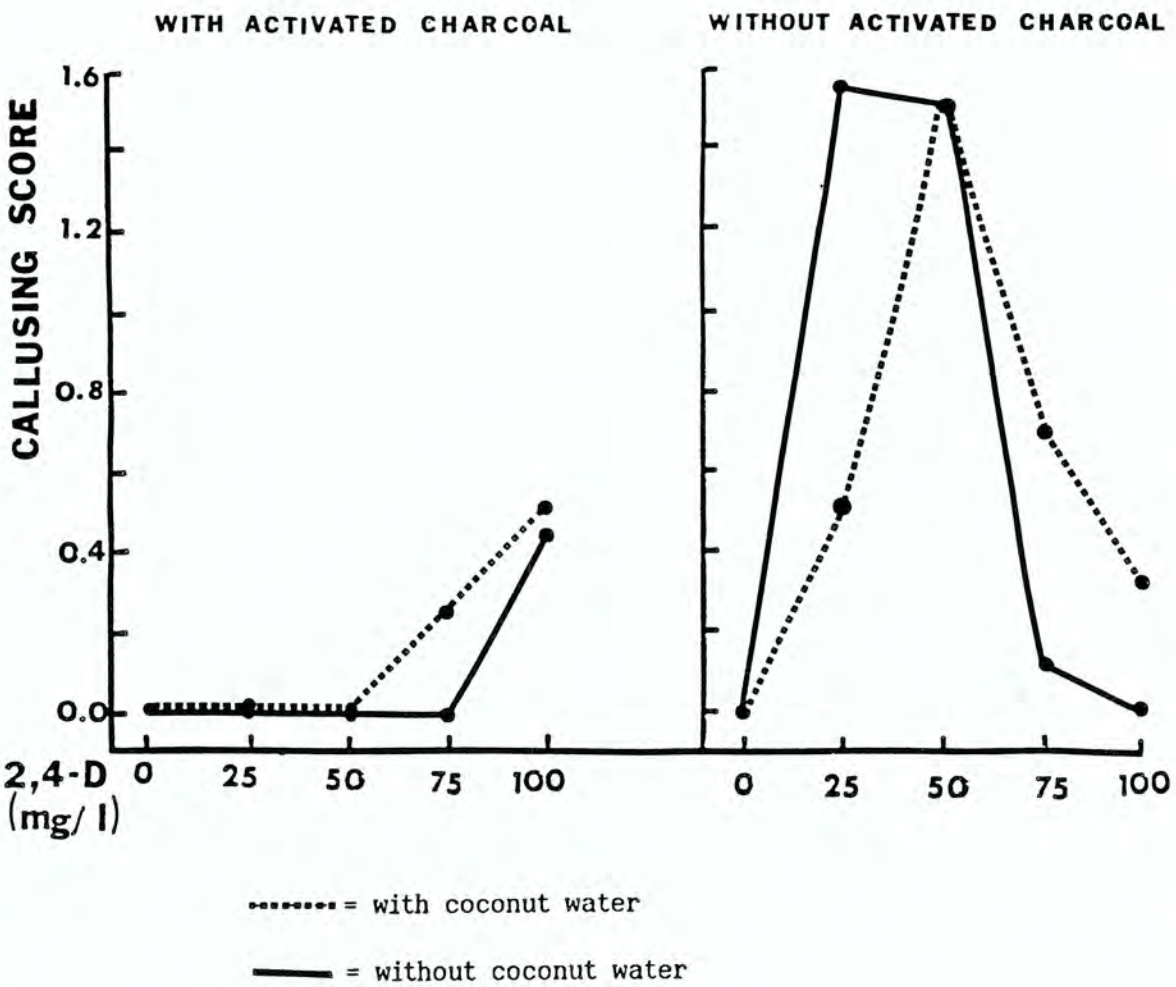


Figure IV-7. Mean callusing scores of pejibaye shoot tips for the interaction among activated charcoal, coconut water, and 2,4-D. Activated charcoal was added at 2.5 g/l, coconut water at 100 ml/l. (Callus scores were assigned as follows: 0 = no callusing; 1 = small areas of callus visible on the explant; 2 = at least 50 % of the explant had callused; and 3 = complete callusing exhibiting vigorous growth. Means for callusing are all lower than 1, because they are the means for the entire experiment, and not just for individual treatments.)

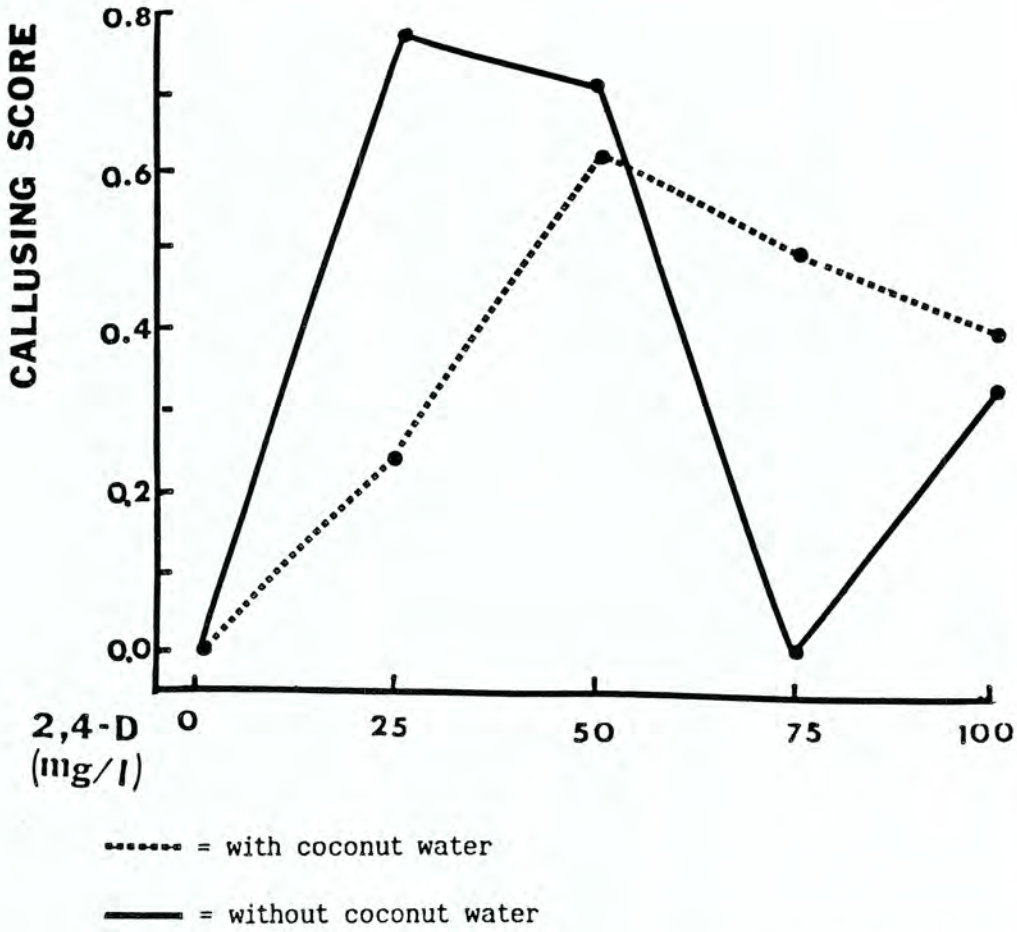


Figure IV-8. Mean callusing scores of pejibaye shoot tips for the interaction between coconut water and 2,4-D. Coconut water was added at 100 ml/l. (Callus scores were assigned as follows: 0 = no callusing; 1 = small areas of callus visible on explant; 2 = at least 50 % of the explant had callused; and 3 = complete callusing exhibiting vigorous growth. Means for callusing are all lower than 1, because they are the means for the entire experiment, and not just for individual treatments.)

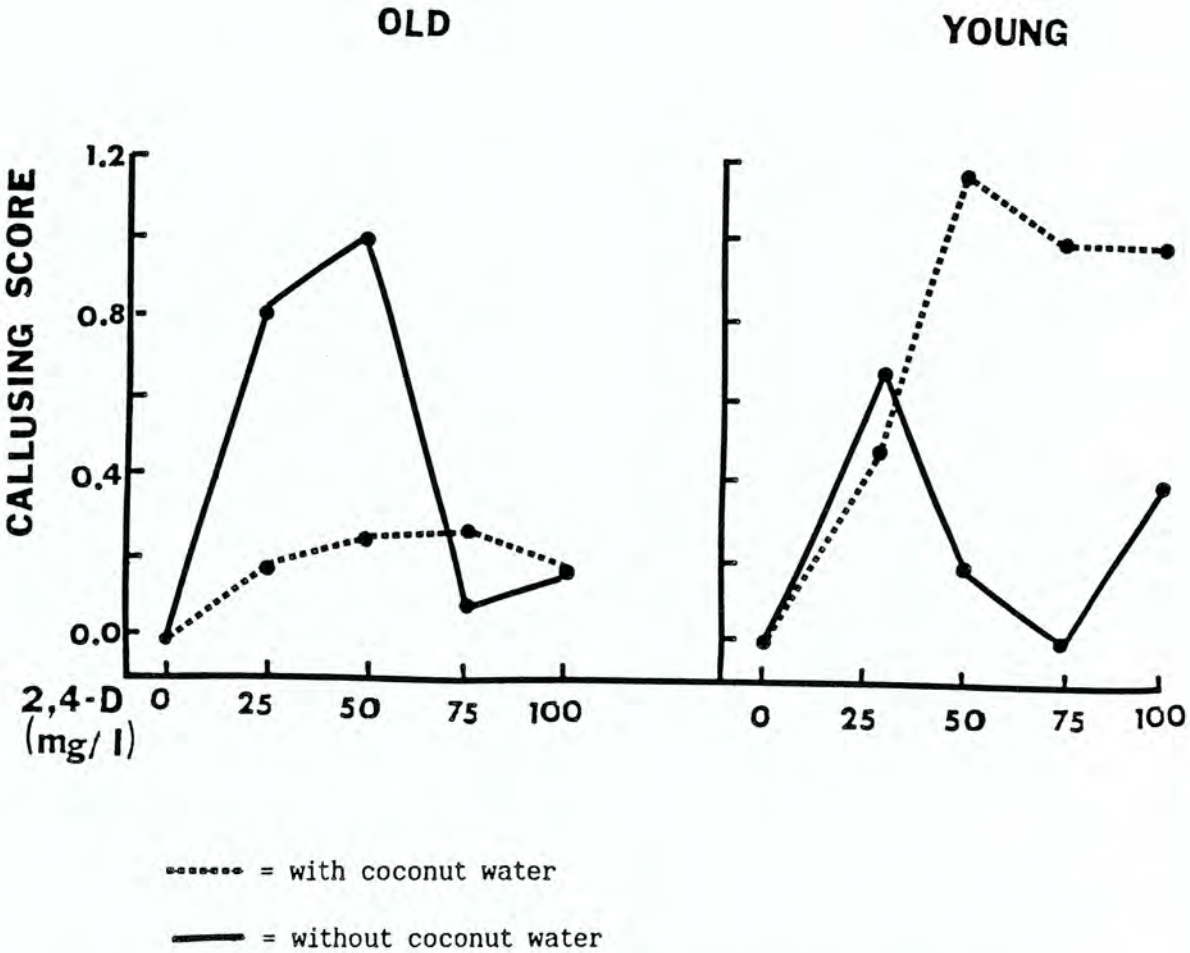


Figure IV-9. Mean callusing scores of pejibaye shoot tips for the interaction among age, coconut water, and 2,4-D. Coconut water was added at 100 ml/l. ("Young" refers to 4-month-old seedlings, "Old" to 1½-year-old seedlings. Callus scores were assigned as follows: 0 = no callusing; 1 = small areas of callus visible on the explant; 2 = at least 50 % of the explant had callused; and 3 = complete callusing exhibiting vigorous growth. Means for callusing are all lower than 1, because they are the means for the entire experiment, and not just for individual treatments.)

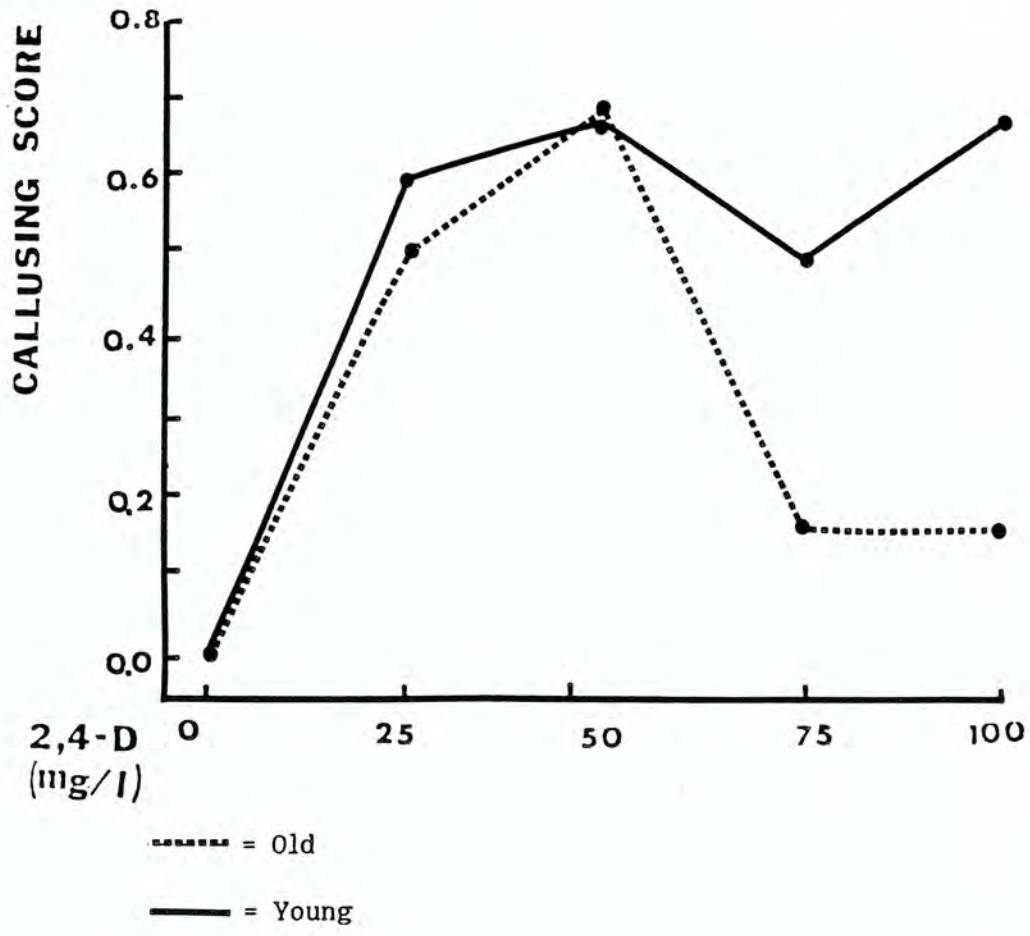


Figure IV-10. Mean callusing scores of pejibaye shoot tips for the interaction between age and 2,4-D. ("Young" refers to 4-month-old seedlings, "Old" to 1½-year-old seedlings. Callus scores were assigned as follows: 0 = no callusing; 1 = small areas of callus visible on explant; 2 = at least 50 % of the explant had callused; and 3 = complete callusing exhibiting vigorous growth. Means for callusing are all lower than 1, because they are the means for the entire experiment, and not just for individual treatments.)

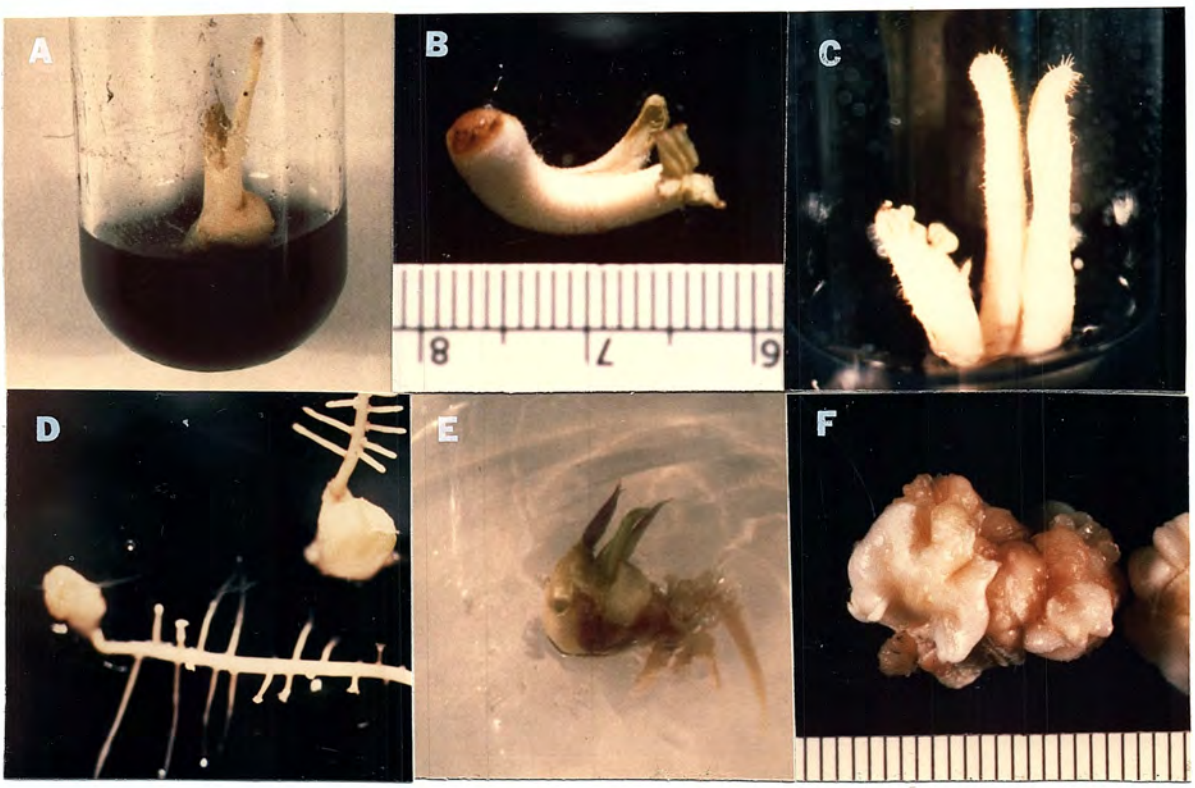


Figure IV-11. Organogenic responses in pejibaye shoot-tip cultures. (a) broadening of the shoot-tip base; (b) shoot-tip elongation with leaflet development; (c) multiple shoot elongation; (d) root development with shoot development suppressed; (e) root and shoot development; and (f) undefined organogenic response

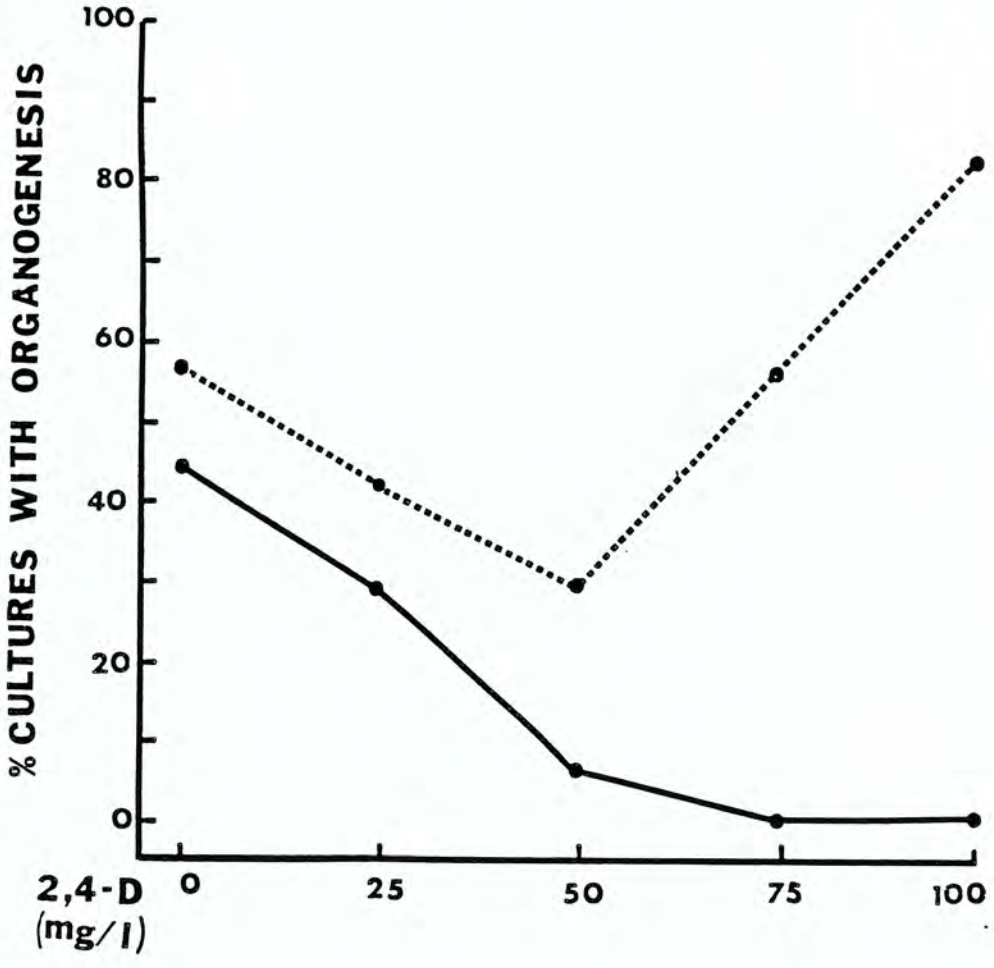
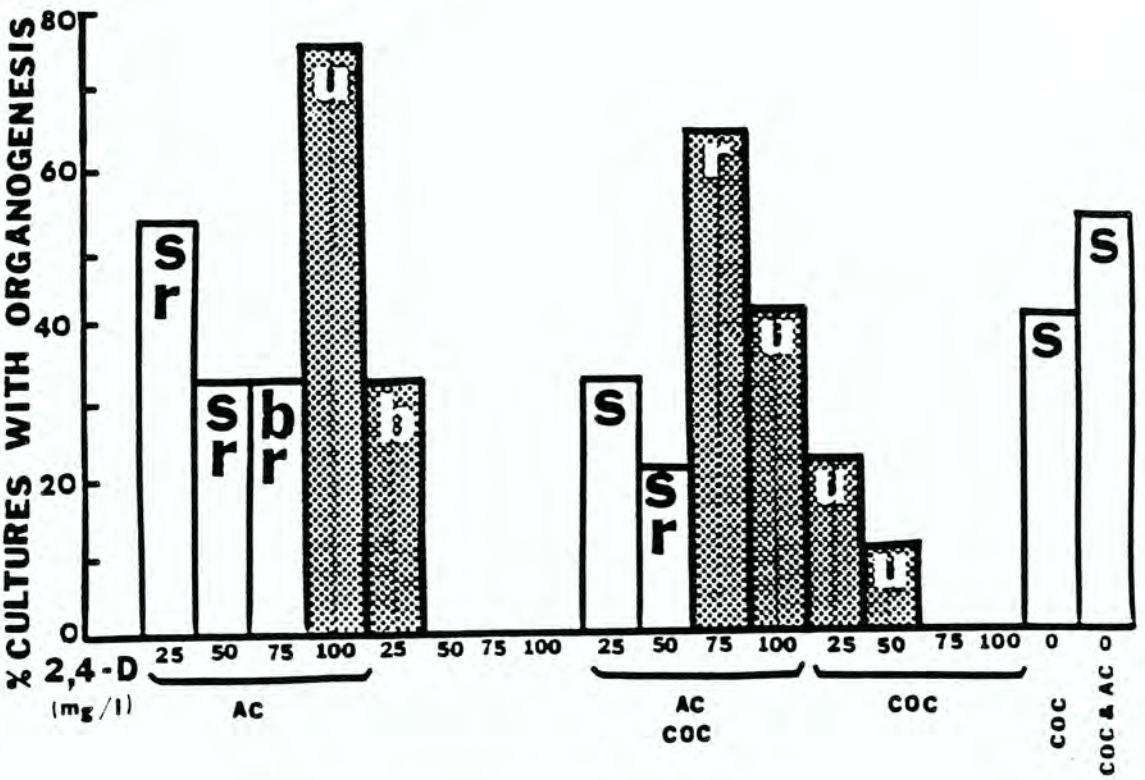


Figure IV-12. Percent pejibaye shoot-tip cultures showing organogenesis for the interaction between activated charcoal and 2,4-D. Activated charcoal was added at 2.5 g/l



= cultures with organogenesis only

= cultures exhibiting both callusing and organogenesis

AC = 2.5 g/l activated charcoal added
COC = 100 ml/l coconut water added
b = broadening of the shoot-tip base
r = root initiation
s = shoot initiation
u = undefined growth

Figure IV-13. Organogenesis rates of pejibaye shoot-tip cultures growing on a variety of media containing five levels of 2,4-D (0, 25, 50, 75, and 100 mg/l) and combinations of coconut water and activated charcoal. All media contained 5 mg/l BA, except when 2,4-D levels were 0, in which case no BA was added either. The organogenic responses indicated in the figure, i.e., broadening of the shoot-tip base, root initiation, shoot initiation, and undefined growth, did not occur exclusively on the treatments indicated, but occurred in at least 70 % of the organogenic cultures

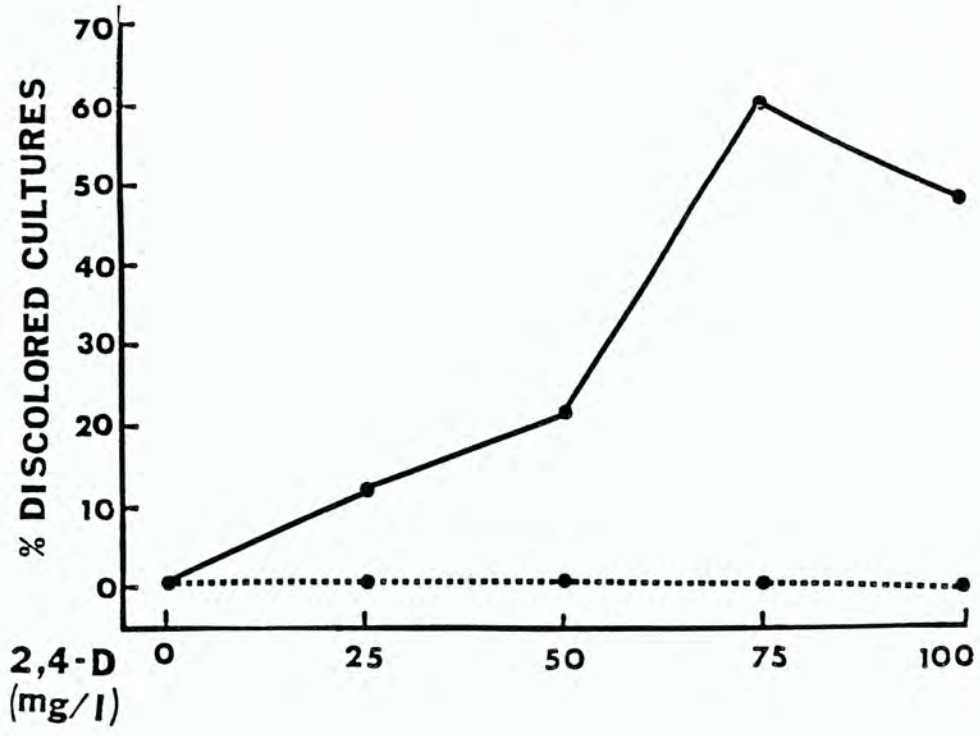


Figure IV-14. Discoloration rate of pejibaye shoot-tip cultures for the interaction between activated charcoal and 2,4-D. Activated charcoal was added at 2.5 g/l

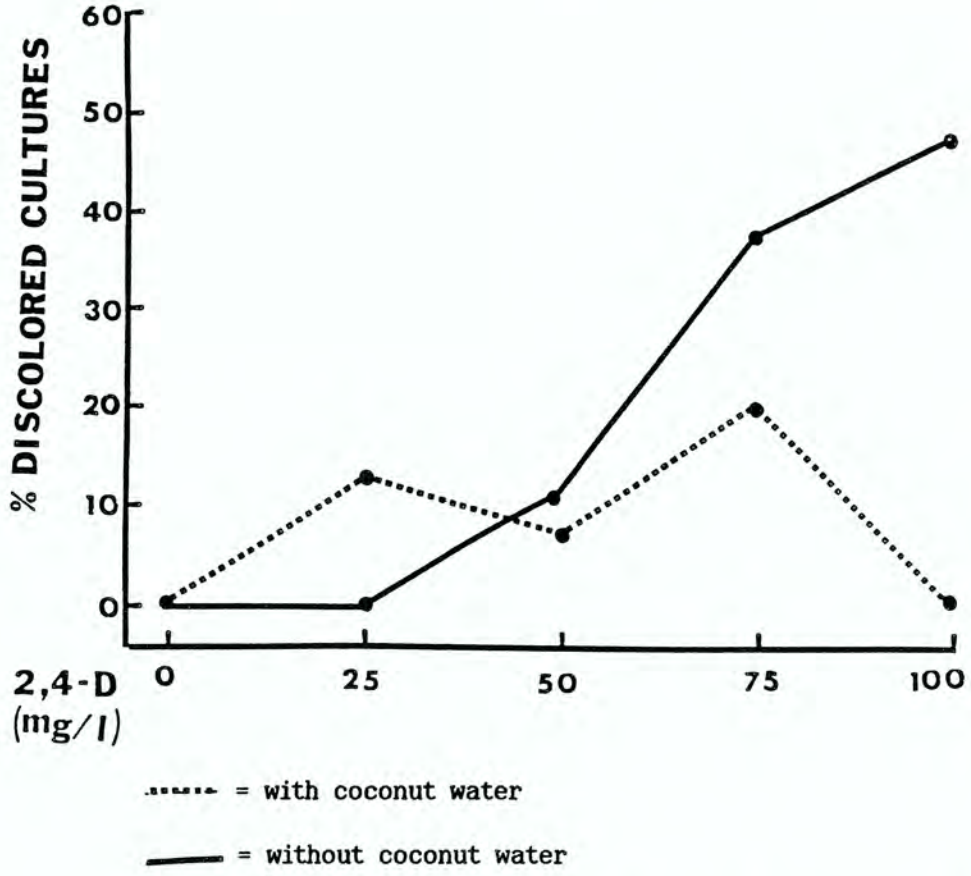


Figure IV-15. Discoloration rate of pejibaye shoot-tip cultures for the interaction between coconut water and 2,4-D. Coconut water was added at 100 ml/l

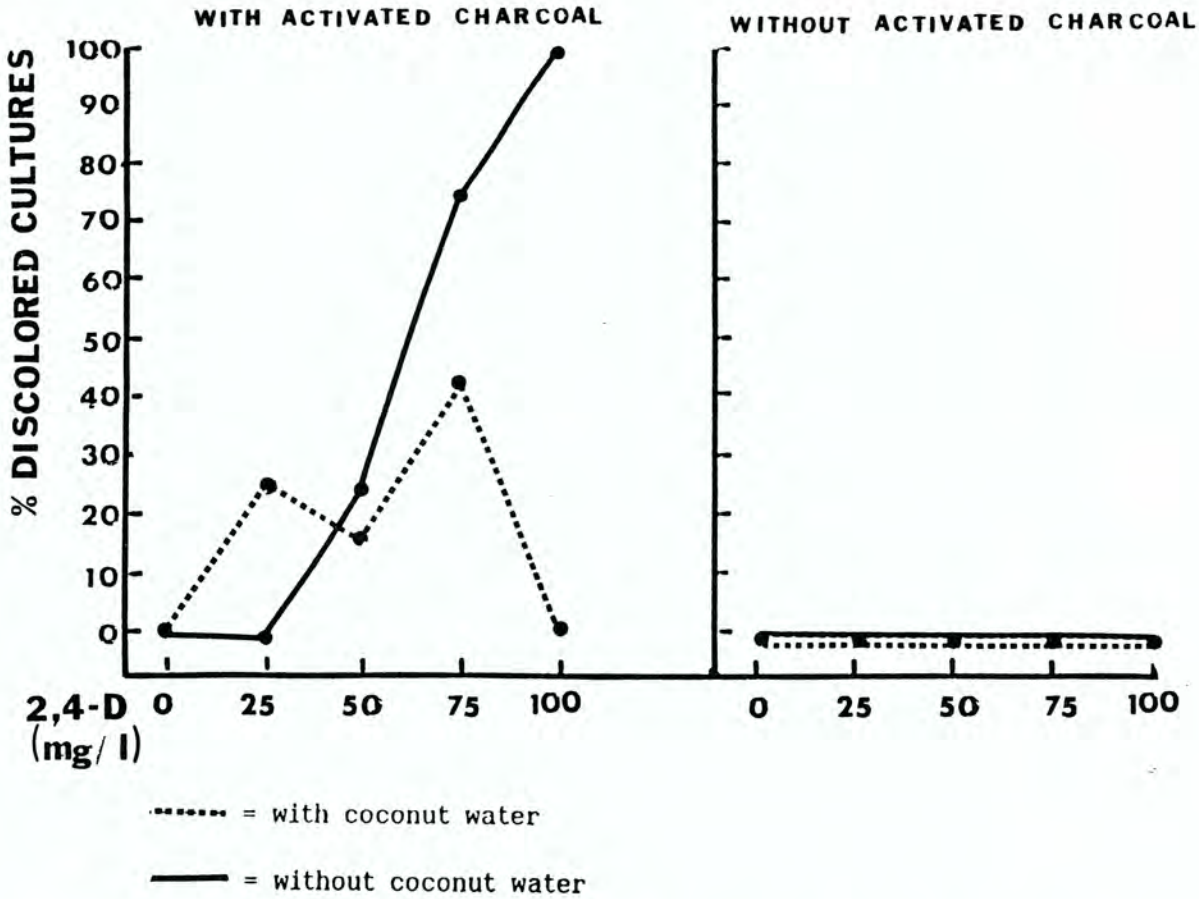


Figure IV-16. Discoloration rate of pejibaye shoot-tip cultures for the interaction among activated charcoal, coconut water, and 2,4-D. Activated charcoal was added at 2.5 g/l, coconut water at 100 ml/l

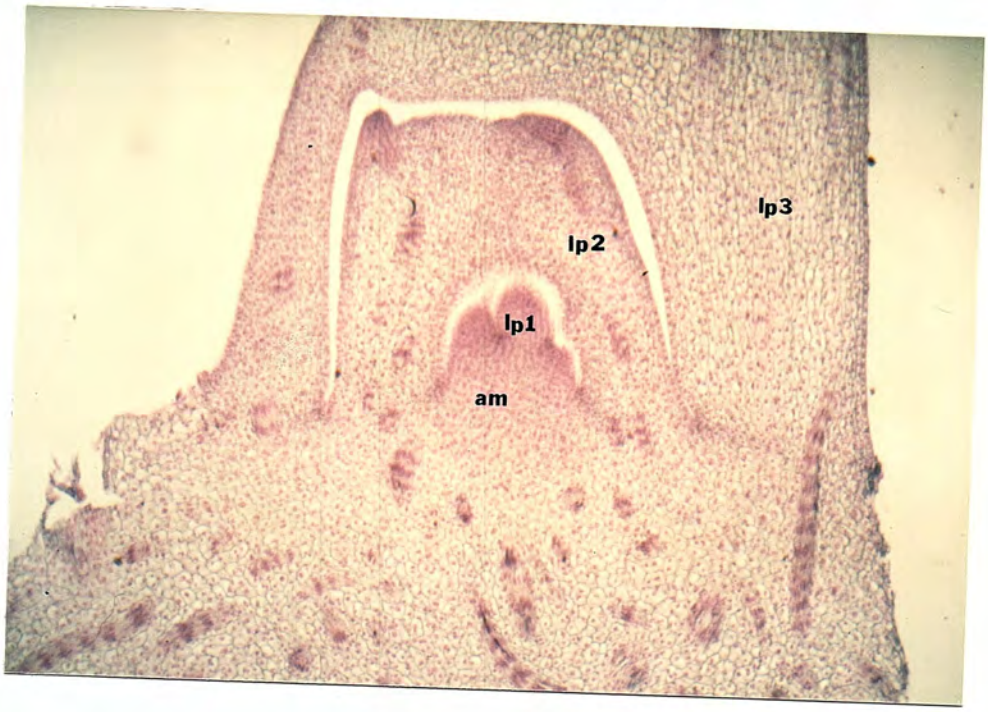


Figure IV-17. Histological section of the base of a pejibaye shoot tip. The apical meristem (am) is enclosed within three leaf primordia (lp1, lp2, and lp3). (Magnification: 20.5x)

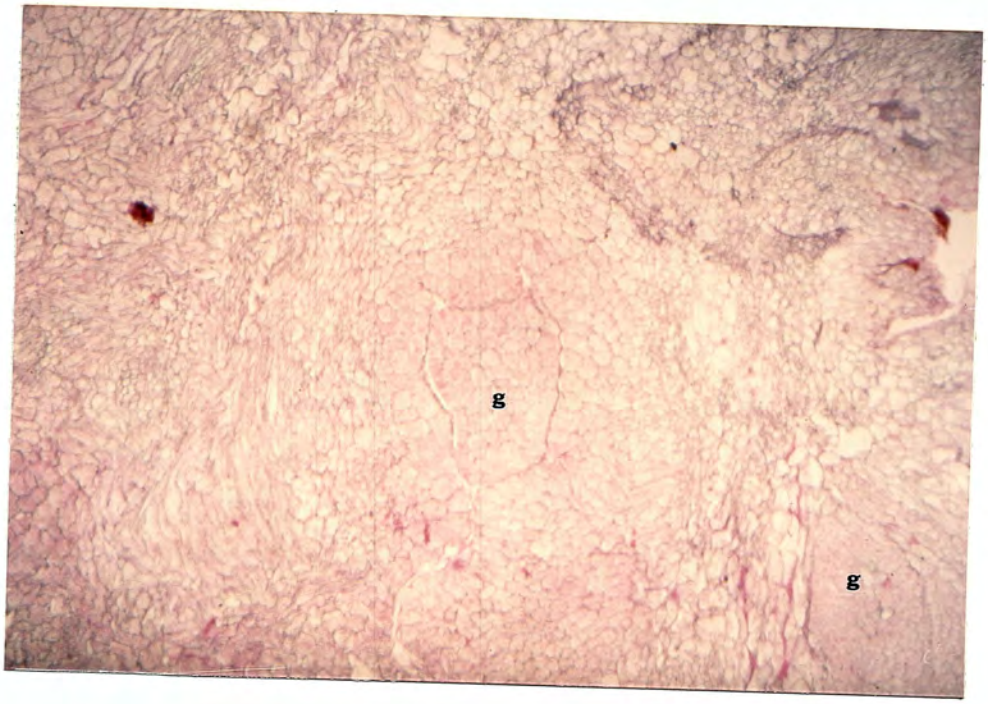


Figure IV-18. Histological slide of a pejibaye shoot-tip callus. The callus was grown on an MS (1962) medium containing 25 mg/l 2,4-D and 5 mg/l BA. Distinct groups of cells (g) are visible in the section. (Magnification: 20.5x)

SUMMARY AND CONCLUSIONS

As is the case in other commercial palm species, the *in vitro* culture of the pejibaye palm is a low-yielding and erratic process when compared with the tissue culture of a dicotyledon such as *Nicotiana tabacum*, which has become a standard subject of tissue culture experimentation. Even though the barriers to reliable palm cloning through tissue culture are still poorly understood, difficult problems in plant physiology have been solved before, and continued research on this topic will produce better results in the future.

This study has contributed to the accumulation of knowledge in the area of pejibaye tissue culture. Encouraging results have been obtained in the tissue culture of subapical stem tissue taken from pejibaye seedlings. The production of offshoots in the pejibaye palm, and the occasional occurrence of adventitious shoot production from stem vascular tissue in nature, provide hope that not only roots, but also shoots will be obtained from subapical stem tissue. A continuation of the research presented here on this topic will provide the answer.

Even though seedlings are unevaluated plants, because their fruit quality and production capacity cannot yet be established, and perhaps also because other phenotypic characteristics cannot be determined so early, the *in vitro* procedures that are successful for seedlings may also prove successful for explants taken from offshoots of adult palms. It is apparently important to use very young seedlings in shoot-tip callus cultures, and an evaluation of the age factor in offshoot cultures is needed. Successful cloning of seedlings is

potentially a very important time-saving tool to be used as part of traditional plant breeding programs.

The seedlings utilized in this experiment proceeded from a randomly mixed lot of seeds which had been collected from several mother plants, and thus were very heterogeneous (Mora-Urpí, 1986, personal communication). The heterogeneity of the seedlings here might resemble the heterogeneity of pejibaye palms growing in their natural habitat, from which explants would be taken for a similar study. Thus, using seedlings from a mixed population approximates a situation in which plant material is taken directly from the field for use in in vitro studies.

Direct organogenesis occurs with relative ease in the pejibaye palm, as indicated by this study and one previous study (Pinedo Panduro, 1987), but it is callus production that lies at the core of rapid cloning, due to its embryogenic potential. Fears that an intermediary callus stage may lead to genetic transformations should be re-evaluated for three reasons. First, previous workers either do not stress the occurrence of genetic aberrations in palms, or indicate that they are non-damaging or non-permanent (Paranjothy, 1986). Second, the pejibaye palm is very heterogeneous in its natural state, so that mutations brought about by in vitro culture are unlikely to produce dramatic variations. In the oil palm, it has already been observed that "variability in clonal populations is less than that in seedling populations" (Corley et al., 1981, in Paranjothy, 1986). Third, somaclonal variation may be desirable, especially if it means finding a dwarf pejibaye type or further spineless varieties.

It is essential for the tropical countries of America to better understand and use their own renewable resources, especially when they have as

much economic potential as the pejibaye palm. Widespread poverty among urban and rural populations, coupled with the destruction of formerly self-sustaining ecosystems on poor soils, demand that more attention be given to nutritious native food crops which have the potential of becoming cash crops as well, and which furthermore are well-adapted to stressful environmental conditions. The pejibaye palm is a species which fulfills all of the above requirements, and it can be grown not just in the wet tropics of America, but also in Asia and Africa (Mora-Urpí et al., 1984), which further underscores its importance.

GENERAL BIBLIOGRAPHY

Abdul, Amir M. 1983. Plant regeneration from callus cultures of Phoenix dactylifera L. Date Palm J. 2(1):57-77.

Abed-Al, Jasim, M. Al-Jibouri, Mohammed A. Kgazal, and Ibrahim S. Al-Saadawi. 1984. Effect of gamma irradiation on pollen germination and pollen tube growth of four male cultivars of date palm (Phoenix dactylifera L.). Pages 589-590 in Novak, F. J., L. Havel, and J. Dolezel, eds. Plant tissue and cell culture application to crop improvement. Czechoslovak Academy of Sciences, Prague, Czechoslovakia.

Ahée, J., P. Arthuis, G. Cas, Y. Duval, G. Guénin, J. Hanower, P. Hanower, D. Lievoux, C. Lioret, B. Malaurie, C. Pannetier, D. Raillot, C. Varéchon, and L. Zuckerman. 1981. La multiplication végétative in vitro du palmier à huile par embryogenèse somatique (Vegetative propagation of the oil palm in vitro by somatic embryogenesis). Oléagineux 36(3):113-118.

Al-Mehdi, A. A., and L. Hogan. 1979. In vitro growth and development of papaya (Carica papaya L.) and date-palm (Phoenix dactylifera L.). Plant Phys. 63(5, Suppl.):100.

Almeida, N. 1980. Cultivation of neglected tropical fruits with promise: The pejibaye. Vegetables for the hot, humid tropics No. 8. United States Department of Agriculture, New Orleans, Louisiana. 10 pp.

Ammar, Seida, and Abd Alatif Ben Badeis. 1983. Vegetative propagation of date palm (Phoenix dactylifera L.) by in vitro culture. Pages 158-166 in Proceedings of the first symposium on the date palm in Saudi Arabia, King Faisal University, 1982. Al-Hassa, Saudi Arabia. (English abstract).

Ammar, Saïda, and Abdellatif Benbadis. 1977. Multiplication végétative du palmier-dattier (Phoenix dactylifera L.) par la culture de tissus de jeunes plantes issues de semis. Compt. Rend. Acad. Sci. Paris, Sér. D 284(18): 1789-1792.

Ammar, Saïda, Abdellatif Benbadis, and Bal Krishna Tripathi. 1987. Floral induction in date palm seedlings (Phoenix dactylifera var. Deglet Nour) cultured in vitro. Can. J. Bot. 65(1):137-142.

Antezana, L. 1972. Palmeras nativas de Bolivia de valor económico. Pages 87-97 in Simposio internacional sobre palmeras de interés económico de la flora amazónica. I.I.C.A., Turrialba, Costa Rica.

Apavatjrut, Pimchai, and J. Blake. 1977. Tissue culture of stem explants of coconut (Cocos nucifera L.). Oléagineux 32(6):267-270.

Arias, Oscar. 1979. Efecto del flurenol en la producción de hijos laterales de pejibaye (Bactris gasipaes H.B.K.). Agron. Costarr. 3(1):47-52.

Arias, Oscar. 1985. Propagación vegetativa por cultivos de tejidos del pejibaye (Bactris gasipaes H.B.K.). ASBANA 24(9):24-27.

Arias M., Oscar, and Fernando Huete V. 1983. Propagación vegetativa in vitro de pejibaye (Bactris gasipaes H.B.K.). Turrialba 33(2):103-108.

Arkcoll, David B., and Jaime P. L. Aguiar. 1984. Peach palm (Bactris gasipaes H.B.K.), a new source of vegetable oil from the wet tropics. J. Sci. Food Agric. 35:520-526

Armstrong, S. W., and D. Francis. 1987. Cell and nuclear area, RNA and protein content, and chromosome counts in meristematic cells of secondary roots and callus of Cocos nucifera. Can. J. Bot. 65(3):547-552.

Attias, M., J. L. Bezerra, D. P. de Oliveira, and W. de Souza. 1987. Ultrastructure of Phytomonas staheli in diseased coconut (Cocos nucifera) and oil palm (Elaeis guineensis). J. Submicrosc. Cytol. 19(1):93-100.

Bah, Assy B. 1986. Culture in vitro d'embryons zygotiques de cocotiers. Oléagineux 41(7):321-326.

Bajaj, Y. P. S. 1984. Induction of growth in frozen embryos of coconut and ovules of citrus. Current Science (India) 53(22):1215-1216.

Balaga, H. Y., and E. V. de Guzman. 1971. The growth and development of coconut "Makapuno" embryos in vitro. II. Increased root incidence and growth in response to media composition and to sequential culture from liquid to solid medium. Philipp. Agric. 53(10):551-565.

Balick, Michael J. 1976. The palm heart as a new commercial crop from tropical America. Principes 20(1):24-28.

Balick, Michael J. 1979. Amazonian oil palms of promise: A survey. Econ. Bot. 33(1):11-28.

Bass, Alice, and William Hughes. 1984. Conditions for isolation and regeneration of viable protoplasts of oil palm (Elaeis guineensis). Plant Cell Rep. 3(5):169-171.

Beauchesne, G. 1983. Vegetative propagation of date palm (Phoenix dactylifera L.) by in vitro culture. Pages 698-700 in Proceedings of the first symposium on the date palm in Saudi Arabia, King Faisal University, 1982. Al-Hassa, Saudi Arabia.

Bhalla-Sarin Neera, Suman Bagga, Sudhir K. Sopory, and Sipra Guha-Mukherjee. 1986. Induction and differentiation of callus from embryos of Cocos nucifera L. by IAA-conjugates. Plant Cell Rep. 5(5):322-324.

Biondi, Stefania, and Trevor A. Thorpe. 1982. Clonal propagation of forest tree species. Pages 197-204 in Rao, A. N., ed. Tissue culture of economically important plants. Proc. Int. Symp., National University of Singapore, Singapore, 1981. The Committee on Science and Technology in Developing Countries (COSTED) and Asian Network for Biological Sciences (SIBiol), Singapore.

Blaak, G. 1980. Vegetative propagation of pejibaye (Bactris gasipaes H.B.K.). Turrialba 30(3):258-261.

Blake, Jennet. 1983. Tissue culture propagation of coconut, date and oil palm. Pages 29-50 in Dodds, John H., ed. Tissue culture of trees. The AVI Publishing Co., Westport, Connecticut.

Blake, Jennet, and C. J. Eeuwens. 1982. Culture of coconut palm tissues with a view to vegetative propagation. Pages 145-148 in Rao, A. N., ed. Tissue culture of economically important plants. Proc. Int. Symp., National University of Singapore, Singapore, 1981. The Committee on Science and Technology in Developing Countries (COSTED) and Asian Network for Biological Sciences (SIBiol), Singapore.

Bonga, J. M. 1982. Vegetative propagation of mature trees by tissue culture. Pages 191-196 in Rao, A. N., ed. Tissue culture of economically important plants. Proc. Int. Symp., National University of Singapore, Singapore, 1981. The Committee on Science and Technology in Developing Countries (COSTED) and Asian Network for Biological Sciences (SIBiol), Singapore.

Boucher, D. H., M. Hansen, S. Risch, and J. H. Vandermeer. 1983. Agriculture. Pages 66-101 in Janzen, Daniel H., ed. Costa Rican natural history. The University of Chicago Press, Chicago, Il.

Brackpool, Avril L., Richard L. Branton, and Jennet Blake. 1986. Regeneration in palms. Pages 207-222 in Vasil, Indra K., ed. Cell culture and somatic cell genetics of plants. Vol. 3. Academic Press, Inc., New York.

Branton, Richard, and Jennet Blake. 1983a. A lovely clone of coconuts. New Scientist 98(1359):554-557.

Branton, R. L., and Jennet Blake. 1983b. Development of organized structures in callus derived from explants of Cocos nucifera L. Ann. Bot. 52(5):673-678.

Burxill, I. H. 1966. A dictionary of economic products of the Malay Peninsula. Vol. 1. Ministry of Agriculture and Co-operatives, Government of Malaysia. 1240 pp.

Butler, Alfred F. 1930. The pejibaye or chontaduro palm; a neglected food plant of Central America. Garden Chronicle 3(87):167.

Calzada, B. J., R. J. Bermúdez, and C. B. Batista. 1977. El pijuayo (Guilielma gasipaes H.B.K. o Bactris gasipaes H.B.K.). Programa de Frutales Nativos, Informativo No. 23. Universidad Nacional Agraria (National Agrarian University), La Molina, Perú.

Camacho, V. E. 1972. El pejibaye (Guilielma gasipaes L.H. Bailey). I.I.C.A., Turrialba, Costa Rica. 17 pp.

Centre for Agriculture Research in Surinam. 1970. Tissue culture of the oil palm (Elaeis guineensis Jacq.). Report for the year 1970. State Agricultural University, Holland. Celos Bulletin 13:10-11.

Centro Agronómico Tropical de Investigación y Enseñanza (CATIE). 1986. Informe del proyecto "Reconocimiento de Nuevas Fuentes de Aceite y Grasa a partir de Palmas Oleaginosas Nativas del Trópico Húmedo Americano". Turrialba, Costa Rica. 71 pp.

Centro de Investigaciones en Productos Naturales. 1986. Aprovechamiento industrial del pejibaye (Bactris gasipaes). Universidad de Costa Rica (University of Costa Rica), San José, Costa Rica. 302 pp.

Chaverri Jiménez, Yolanda. 1984. Efecto de diferentes fuentes y niveles de nitrógeno en el medio nutritivo para el cultivo in vitro de ápices de pejibaye (Bactris gasipaes H.B.K.). Master's Thesis. Universidad de Costa Rica (University of Costa Rica), San José, Costa Rica. 63 pp.

Cheldelin, V. H., and R. J. Williams. 1942. Adsorption of organic compounds. I. Adsorption of ampholytes on an activated charcoal. J. Am. Chem. Soc. 64:1513-1516.

Cheng, Y. W., and S. E. Chua. 1982. The use of air flow system in plant tissue and organ cultures. Pages 210-212 in Rao, A. N., ed. Tissue culture of economically important plants. Proc. Int. Symp., National University of Singapore, Singapore, 1981. The Committee on Science and Technology in Developing Countries (COSTED) and Asian Network for Biological Sciences (SIBiol), Singapore.

Chin, H. F., and E. H. Roberts. 1980. Recalcitrant crop seeds. Tropical Press SDN. BHD, Kuala Lumpur, Malaysia. 152 pp.

Clement, Charles R., and Ana Ma. Arias de Guerrero. 1983. Pejibaye (Bactris gasipaes): Bibliografía parcialmente anotada (Partially annotated bibliography). Documentación e Información Agrícola No. 121. Programa para el Desarrollo del Trópico Americano, I.I.C.A.-TROPICOS, Turrialba, Costa Rica. 68 pp.

Clement, Carles R., and Instituto Nacional de Pesquisas da Amazonia. 1986. Bactris gasipaes. Lista mínima de descriptores para el banco de germoplasma. Comité Coordinador Internacional para la Investigación sobre Bactris gasipaes H.B.K., Costa Rica. 15 pp.

Clement, Charles R., and Jorge Mora Urpí. 1982. The pejibaye palm (Bactris gasipaes) comes of age. Principes 26(3):150-152.

Clement, Charles R., and Jorge Mora Urpí. 1983. Leaf morphology of the pejibaye palm (Bactris gasipaes H.B.K.). Rev. Biol. Trop. 31(1):103-112.

Cochran, William G., and Gertrude M. Cox. 1957. Experimental designs. John Wiley and Sons, New York. 617 pp.

Constantin, M. J., R. R. Henke, and M. A. Mansur. 1977. Effect of activated charcoal on callus growth and shoot organogenesis in tobacco. In Vitro 13(5):293-296.

Corley, R. H. V. 1976. Sex differentiation in oil palm: Effects of growth regulators. J. Exp. Bot. 27(98):553-558.

Corley, R. H. V., J. M. Barrett, and L. H. Jones. 1977. Vegetative propagation of oil palm via tissue culture. Oil Palm News 22:2-7.

Corley, R. H. V., K. C. Wooi, and C. Y. Wong. 1979. Progress with vegetative propagation of oil palm. Planter 55(641):377-380.

Cox, D. F. 1983. Planning, executing, and interpreting experiments. Unpublished notes. Department of Statistics, Iowa State University, Ames, Iowa. 437 pp.

Cui, Y. F., Z. Gong, X. H. Chen, X. D. Shao, and Z. Q. Zhu. 1985. Induction of embryoids and plantlets from oil palm callus. Genetic Manipulation in Crops Newsletter (GMICN) 1(1):40-41.

Cui, Y. F., Z. Gong, X. H. Chen, X. T. Shao, and Z. Q. Zhu. 1986. Callus induction and plant regeneration from embryo of Elaeis guineensis Jacq. Acta Bot. Sin. 28(6):582-588.

Cutter, V. M., Jr., and Katherine S. Wilson. 1954. Effect of coconut endosperm and other growth stimulants upon the development in vitro of embryos of Cocos nucifera. Bot. Gaz. 115:234-240.

D'Amato, F. 1978. Chromosome number variation in cultured cells and regenerated plants. Pages 287-295 in Thorpe, T. A., ed. Frontiers of plant tissue culture. International Association of Plant Tissue Culture, Calgary, Canada.

Davis, T. A. 1969. Clonal propagation of the coconut. World Crops 21:253-255.

Davis, T. Antony. 1978. Some unusual formations in palms. Principes 23(2):80-83.

Davis, T. A., H. Sudasrip, and H. Azis. 1981. Bulbil-shoot production from clonally propagated coconuts. Principes 25(3):124-129.

Davis, T. Antony, H. Sudasrip, and H. Azis. 1982. Propagation of the coconut. Pages 302-303 in Rao, A. N., ed. Tissue culture of economically important plants. Proc. Int. Symp., National University of Singapore, Singapore, 1981. The Committee on Science and Technology in Developing Countries (COSTED) and Asian Network for Biological Sciences (SIBiol), Singapore.

Dawe, M. T. 1928. Branching in the African oil palm. Kew Bull. 42:21-22.

Debergh, P. C. 1983. Effects of agar brand and concentration on the tissue culture medium. Physiol. Plant. 59:270-276.

de Guzman, E. V. 1971. The growth and development of coconut 'Makapuno' embryo in vitro. Philipp. Agric. 53:65-78.

de Guzman, E. V., and A. G. del Rosario. 1964. The growth and development of Cocos nucifera L. 'Makapuno' embryo in vitro. Philipp. Agric. 48(2-3):82-94.

de Guzman, E. V., and G. C. Manuel. 1977. Improved root growth in embryo and seedling cultures of coconut Makapuno by the incorporation of charcoal in the growth medium. Philipp. J. Coconut Stud. 2:35-39.

de Guzman, E. V., A. G. del Rosario, and E. C. Eusebio. 1971. The growth and development of coconut "Makapuno" embryo in vitro. III. Resumption of root growth in high sugar media. Philipp. Agric. 53(10):566-579.

de Guzman, E. V., A. G. del Rosario, and E. M. Ubalde. 1978. Proliferative growths and organogenesis in coconut embryo and tissue cultures. Philipp. J. Coconut Stud. 3:1-10.

del Rosario, A. G., and E. V. De Guzman. 1976. The growth of coconut 'Makapuno' embryos in vitro as affected by mineral composition and sugar level of the medium during liquid and solid cultures. Philipp. J. Sc. 105(4):215-222.

del Rosario, A. G., and E. V. De Guzman. 1982. The status of plant tissue culture in the Philippines. Pages 293-294 in Rao, A. N., ed. Tissue culture of economically important plants. Proc. Int. Symp., National University of Singapore, Singapore, 1981. The Committee on Science and Technology in Developing Countries (COSTED) and Asian Network for Biological Sciences (SIBiol), Singapore.

DeMason, Darleen A. 1985. Histochemical and ultrastructural changes in the haustorium of date (Phoenix dactylifera L.). Protoplasma 126:168-177.

DeMason, Darleen A., and B. Tisserat. 1980. The occurrence and structure of apparently bisexual flowers in the date palm, Phoenix dactylifera L. (Arecaceae). Bot. J. Linn. Soc. 81(4):283-292.

Dierolt, C. 1978. Comentarios sobre posibles diseños de equipo para cosecha y beneficio del chontaduro. Pages 34-39 in Serie Informativo No. 1. Secretaría de Agricultura y Fomento, Cali, Colombia.

Dix, L., and J. van Staden. 1982. Auxin and gibberellin-like substances in coconut milk and malt extract. Plant Cell Tiss. Org. Cult. 1(4):239-246.

Douglas, Richard. 1982. Notes on culture: Get those palms in the ground. Principes 26(3):152-153.

Drew, R. L. K. 1979. Effect of activated charcoal on embryogenesis and regeneration of plantlets from suspension cultures of carrot (Daucus carota L.). Ann. Bot. 44:387-389.

Drira, N. 1983. Multiplication végétative du palmier dattier (Phoenix dactylifera L.) par la culture in vitro de bourgeons axillaires et de feuilles qui en dérivent. Compt. Rend. Acad. Sci. Sér. III 296:1077-1082.

Drira, Noureddine, and Abdellatif Benbadis. 1985. Multiplication végétative du palmier dattier (Phoenix dactylifera L.) par réversion en culture in vitro d'ébauches florales de pieds femelles (Vegetative mutliplication of date palm [Phoenix dactylifera L.] by reversion of in vitro cultured female flower buds). J. Plant Phys. 119:227-235.

Duncan, David R., and Jack M. Widholm. 1986. Cell selection for crop improvement. Pages 153-173 in Janick, Jules, ed. Plant breeding reviews, Vol. 4. The AVI Publishing Company, Inc., Westport, Connecticut.

Eeuwens, C. J. 1976. Mineral requirements for growth and callus initiation of tissue explants excised from mature coconut palms (Cocos nucifera) and cultured in vitro. Physiol. Plant. 36(1):23-28.

Eeuwens, C. J. 1978. Effects of organic nutrients and hormones on growth and development of tissue explants from coconut (Cocos nucifera) and date (Phoenix dactylifera) palms cultured in vitro. Physiol. Plant. 42(2):173-178.

Eeuwens, C. J., and J. Blake. 1977. Culture of coconut and date palm tissue with a view to vegetative propagation. Acta Horticulturae 78:277-286.

El-Hennawy, Hussein M., and Yussuf A. Wally. 1980. Vegetative propagation of date palm Phoenix dactylefera [sic] L. by explant culture in vitro. Egypt. J. Hort. 7(2):211-220.

El-Hennawy, Hussein M., and Yussuf A. Wally. 1978. Date palm Phoenix dactylifera bud differentiation in vitro. Egypt. J. Hort. 5(1):81-82

Erdelsky, Karol, and Zdena Cholvadtova. 1970. Wirkung von Kokosmilch und Hefeextrakt auf das Wachstum von Gewebekulturen der Tabakpflanze Nicotiana tabacum L. (The effect of coconut milk and yeast extract on the growth of tissue culture of tobacco Nicotiana tabacum L.). Acta Fac. Rerum. Nat. Comenianae Physiol. Plant. 1:99-116. (English summary).

Essig, Frederick B. 1971. Observations on pollination in Bactris. Principes 15:20-24.

Evans, David A., William R. Sharp, Philip V. Ammirato, and Yasuyuki Yamada, eds. 1983. Handbook of plant cell culture. Vol. 1. Macmillan Publishing Company, New York.

Finkle, B. J., J. M. Ulrich, D. W. Rains, B. B. Tisserat, and G. W. Schaeffer. 1979. Survival of alfalfa, rice, and date palm callus after liquid nitrogen freezing. Cryobiology 16(6):583. Abstract.

Fisher, Jack B., and James H. Tsai. 1978. In vitro growth of embryos and callus of coconut palm. In Vitro 14(3):307-311.

Fisher, Jack B., and James H. Tsai. 1979. A branched coconut seedling in tissue culture. Principes 23(3):128-131.

Fridborg, Gunnar, and Tage Eriksson. 1975. Effects of activated charcoal on growth and morphogenesis in cell cultures. Physiol. Plant. 34:306-308.

Fridborg, Gunnar, Marianne Pedersén, Lars-Erik Landström, and Tage Eriksson. 1978. The effect of activated charcoal on tissue cultures: Adsorption of metabolites inhibiting morphogenesis. Physiol. Plant. 43(2):104-106.

Gabr, M. F., and B. Tisserat. 1984. Parameters involved in the isolation, culture cell wall regeneration, and callus formation from palm (Phoenix dactylifera) and carrot (Daucus carota) protoplasts. Date Palm J. 3(2):359-366.

Gabr, Mahdia F., and Brent Tisserat. 1985. Propagating palms in vitro with special emphasis on the date palm (Phoenix dactylifera L.). Scientia Horticulturae 25(3):255-262.

Gupta, P. K., S. V. Kendurkar, V. M. Kulkarni, M. V. Shirgurkar, and A. F. Mascarenhas. 1984. Somatic embryogenesis and plants from zygotic embryos of coconut (Cocos nucifera L.) in vitro. Plant Cell Rep. 3(6):222-225.

Haccius, Barbara, and V. J. Philip. 1979. Embryo development in Cocos nucifera: A critical contribution to a general understanding of palm embryogenesis. Plant Syst. Evol. 132(1-2):91-106.

Haibou, T. K., and A. Kovoor. 1982. Regeneration of callus from coconut protoplasts. Pages 149-151 in Rao, A. N., ed. Tissue culture of economically important plants. Proc. Int. Symp., National University of Singapore, Singapore, 1981. The Committee on Science and Technology in Developing Countries (COSTED) and Asian Network for Biological Sciences (SIBiol), Singapore.

Halperin, W. 1970. Embryos from somatic plant cells. Pages 169-191 in Padykula, H. A., ed. Control mechanisms in the expression of cellular genotypes. Academic Press, Inc., New York.

Halperin, Walter. 1986. Attainment and retention of morphogenetic capacity in vitro. Pages 3-47 in Vasil, Indra K., ed. Cell culture and somatic cell genetics of plants. Vol. 3. Academic Press, Inc., New York.

Hammond, Earl G., Wenchi P. Pan, and Jorge Mora-Urpí. 1982. Fatty acid composition and glyceride structure of the mesocarp and kernel oils of the pejibaye palm (Bactris gasipaes H.B.K.). Rev. Biol. Trop. 30(1):91-93.

Hanower, J., and P. Hanower. 1984. Inhibition et stimulation, en culture in vitro, de l'embryogenèse des souches issues d'explants foliaires de palmier à huile. Comp. Rend. Acad. Sci., Sér. III 298(2):45-48.

Hartmann, Hudson T., and Dale E. Kester. 1983. Plant propagation. 4th edition. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. 727 pp.

Hodel, Don. 1977. Notes on embryo culture of palms. Principes 21:103-108.

Huete V., F., and O. Arias M. 1981. Propagación vegetativa del pejibaye. ASBANA 5(14):10-13.

Huete V., Fernando, Oscar Arias, and Juan José Alán. 1981. Evaluación de medios de cultivo y concentraciones hormonales para la propagación vegetativa in vitro de pejibaye (Bactris gasipaes H.B.K.). Pages 23-34 in Resúmenes de las Primeras Jornadas de Investigación, Universidad de Costa Rica (University of Costa Rica), San José, Costa Rica.

Hunault. G. 1979. Recherches sur le comportement des fragments d'organes et des tissus de monocotyledones cultivés in vitro. Rev. Cytol. Biol. Veget. Bot. 2:259-287.

Hunter, J. Robert. 1969. The lack of acceptance of the pejibaye palm and a relative comparison of its productivity to that of maize. Econ. Bot. 23:237-244.

Ignacio, L. F., Jr. 1985. Present and future position of coconut oil in world supply and trade. JAOCS (J. Am. Oil Chem. Soc.) 62(2):197-203.

Instituto Vallecaucano de Investigaciones Científicas (INCIVA). 1981. Usos culinarios del chontaduro. INCIVA, Cali, Colombia. 76 pp.

Iyer, R. D. 1982. Embryo and tissue culture for crop improvement, especially of perennials, germplasm conservation and exchange. Pages 219-230 in Rao, A. N., ed. Tissue culture of economically important plants. Proc. Int. Symp., National University of Singapore, Singapore, 1981. The Committee on Science and Technology in Developing Countries (COSTED) and Asian Network for Biological Sciences (SIBiol), Singapore.

James, A. T. 1984. Plant tissue culture: Achievements and prospects. Proc. Roy. Soc. Lon., B. 222(1227):135-145.

James, A. T. 1985. The biotechnology of oilseed crops. JAOCS (J. Am. Oil Chem. Soc.) 62(2):204-206.

Janos, David P. 1977. Vesicular-arbuscular mycorrhizae affect the growth of Bactris gasipaes. Principes 21:12-18.

Janzen, Daniel H., ed. 1983. Costa Rican natural history. The University of Chicago Press, Chicago, Illinois. 816 pp.

Johannessen, Carl L. 1966a. Pejibayes in commercial production. Turrialba 16(2):181-187.

Johannessen, Carl L. 1966b. Pejibaye palm: Yields, prices and labor costs. Econ. Bot. 20:302-315.

Johannessen, Carl L. 1967. Pejibaye palm: Physical and chemical analysis of the fruit. Econ. Bot. 21:371-378.

Johansen, Donald Alexander. 1940. Plant microtechnique. McGraw-Hill, New York. 523 pp.

Jones, L. H. 1974. Propagation of clonal oil palms by tissue culture. Oil Palm News 17:1-8.

Jones, L. H. 1980. Clonal oil palm. Unilever Research, Colworth Laboratory, U. K. 13 pp.

Jones, L. H. 1983. The oil palm and its clonal propagation by tissue culture. Biologist 30(4):181-188.

Karunaratne, S., C. Kurukulaarachchi, and C. Gamage. 1985. A report on the culture of embryos of dwarf coconut, Cocos nucifera L. var. nana in vitro. Cocos 3:1-8.

Khalil, M. S., M. A. Khan, and M. S. Al-Kahtani. 1983. In vitro embryo culture of date palm (Phoenix dactylifera L.). Pages 142-150 in Proceedings of the first symposium on the date palm in Saudi Arabia, King Faisal University, 1982. Al-Hassa, Saudi Arabia.

Khan, M. A., M. S. Khalil, and M. S. Al-Kahtani. 1983. In vitro culture of different tissues of date palm (Phoenix dactylifera L.) offshoot. Pages 152-157 in Proceedings of the first symposium on the date palm in Saudi Arabia, King Faisal University, 1982. Al-Hassa, Saudi Arabia.

Kohlenbach, H. W., and W. Wernicke. 1978. Investigations on the inhibitory effect of agar and the function of active carbon in anther culture. Z. Pflanzenphysiol. 86:463-472.

Kovoor, A. 1981. Palm tissue culture: State of the art and its application to the coconut. FAO Plant Production and Protection Paper No. 30. Food and Agriculture Organization of the United Nations, Rome.

Krikorian, A. D., and Robert P. Kann. 1986. Oil palm improvement via tissue culture. Pages 175-202 in Janick, Jules, ed. Plant breeding reviews. Vol. 4. Avi Publishing Company, Inc., Westport, Connecticut.

Kumar, P. Prakasch, C. R. Raju, Mini Chandramohan, and R. D. Iyer. 1985. Induction and maintenance of friable callus from the cellular endosperm of Cocos nucifera L. Plant Sci. (Ireland) 40(3):203-207.

Kuruvinashetti, M. S., and R. D. Iyer. 1982. An evaluation of tissue culture techniques in coconut and turmeric. Pages 101-106 in Vishveshwara, S., ed. Genetics, plant breeding and horticulture. Proc. Fourth Annual Symp. on Plantation Crops (PLACROSYM IV), Central Food Technological Research Institute, Mysore, India, 1981. PLACROSYM Standing Committee, India.

Kyte, L. 1983. Plants from test tubes. Timber Press, Portland, Oregon. 132 pp.

Larkin, P. J. 1987. Somaclonal variation: History, method, and meaning. Iowa State Journal of Research 61(4):393-434.

Leary, John V., Nels Nelson, Brent Tisserat, and Elizabeth A. Allingham. 1986. Isolation of pathogenic Bacillus circulans from callus cultures and healthy offshoots of date palm (Phoenix dactylifera L.). Appl. Environ. Microbiol. 52(5):1173-1176.

Letham, D. S. 1974. Regulators of cell division in plant tissues. XX. The cytokinins of coconut milk. Physiol. Plant. 32:66-70.

Lioret, C. 1981. Vegetative propagation of oil palm by somatic embryogenesis. Pages 163-172 in Pushpavajah, R. and P. S. Chew, eds. Oil palm in the eighties. Vol. 1. Incorporated Society of Planters, Mysore, India.

Lioret, C., and M. Ollagnier. 1981. La culture in vitro de tissus chez le palmier à huile (The culture of oil palm tissues in vitro). Oléagineux 36(3):111-112. (English translation).

Lunderstädt, J. 1976. Isolation and analysis of plant phenolics from foliage in relation to species characterization and to resistance against insetcs and pathogens. Pages 158-164 in Miksche, J. P., ed. Modern methods in forest genetics. Springer-Verlag, Berlin, West Germany.

Martin, J. P., S. Cas, and H. Rabéchault. 1972b. Note préliminaire sur la culture in vitro de racines de palmier à huile (Elaeis guineensis Jacq. var. Dura Becc. et son hybride Dura x var. Pisifera Becc.) (Aseptic cultures of oil palm roots (Elaeis guineensis var. Dura Becc. and its hybrid x var. Pisifera Becc.). Oléagineux 27(6):303-305. (English summary).

Mater, A. A. 1983. Plant regeneration from callus cultures of Phoenix dactylifera L. Date Palm Journal 2:57-77.

Mathias, Raymond J. and Eric S. Simpson. 1986. The interaction of genotype and culture medium on the tissue culture responses of wheat (Triticum aestivum L. em. thell) callus. Plant Cell Tiss. Org. Cult. 7(1):31-37

Mielke, S. 1985. Present and future position of palm and palm kernel oils in world supply and trade. JAOCS (J. Am. Oil Chem. Soc.) 62(2):193-197.

Ministerio de Desarrollo Agropecuario y Reforma Agraria, Dirección General de Agricultura, Dirección de Trópico Húmedo (Nicaragua). 1986. 1) Reconocimiento y estudio de nuevas fuentes de aceite y grasa a partir de palmas oleaginosas nativas del trópico húmedo americano. 2) Estudio biométrico del pijibay (Bactris gasipaes H.B.K.). Presented by Ing. Julio Rothschuh, Programa Nacional de Palmas (National Palm Program), as part of the proyect "Reconocimiento y Estudio de Nuevas Fuentes de Aceite y Grasa a Partir de Palmas Oleaginosas Nativas del Trópico Húmedo Americano". Manuscript. 18 pp.

Moll, H. A. J. 1987. The economics of oil palm. Centre for Agricultural Publishing and Documentation (Pudoc), Wageningen, The Netherlands. 288 pp.

Monfort, S. 1985. Androgenesis of coconut: Embryos from anther culture. Z. Pflanzenzüchtg. 94:251-254.

Montenegro, Herman M. 1985. Coconut oil and its byproducts. JAOCS (J. Am. Oil Chem. Soc.) 62(2):259-261.

Moore, Harold E., Jr. 1982. Publications on palms. Principes 26(4):182-185.

Mora-Urpí, Jorge. 1978. Pejibaye: Consideraciones sobre algunos proyectos en marcha. ASBANA 3(7):5-6.

Mora Urpí, Jorge. 1979. Consideraciones sobre el posible origen del pejibaye cultivado. ASBANA 3:5,14-15.

Mora-Urpí, Jorge. 1980. Método práctico para germinación de semillas de pejibaye. ASBANA 3(10):14-15.

Mora-Urpí, J. 1981. El ciclo de floración en pejibaye (Bactris gasipaes H.B.K.) y su posible manejo agronómico. Agron. Costarr. 5(1/2):115-119.

Mora Urpí, Jorge. 1982a. Composición química del aceite de pejibaye. ASBANA 6(18):19-20.

Mora Urpí, Jorge. 1982b. Polinización en Bactris gasipaes H.B.K. (Palmae): Nota adicional. Rev. Biol. Trop. 30(2):174-176.

Mora Urpí, Jorge. 1983. El pejibaye (Bactris gasipaes H.B.K.): Origen, biología floral y manejo agronómico. Pages 118-160 in Palmeras poco utilizadas de América tropical. Reunión de consulta. (Proceedings of the workshop on underutilized palms of tropical America, 1983, Turrialba, Costa Rica). FAO. Vol. 43. FAO/CATIE, San José, Costa Rica.

Mora Urpí, Jorge, and Charles R. Clement. 1981. Aspectos taxonómicos relativos al pejibaye (Bactris gasipaes H.B.K.). Rev. Biol. Trop. 29(1):139-142.

Mora Urpí, Jorge, and Emilia María Solís. 1980. Polinización en Bactris gasipaes H.B.K. (Palmae). Rev. Biol. Trop. 28(1):153-174.

Mora Urpí, J., E. Vargas, C. A. López, M. Villaplana, G. Allón, and C. Blanco. 1984. The pejibaye palm (Bactris gasipaes H.B.K.). Food and Agriculture Organization of the United Nations, San José, Costa Rica. 16 pp.

Morán, R. M. J. 1983. Posibilidad de utilizar las técnicas in vitro en algunas palmeras de la Amazonía peruana. Pages 161-162 in Palmeras poco utilizadas de América tropical. Renuión de consulta. (Proceedings of the workshop on underutilized palms of tropical America, 1983, Turrialba, Costa Rica). FAO. Vol. 43. FAO/CATIE, San José, Costa Rica.

Murashige, Toshio. 1974. Plant propagation through tissue cultures. Ann. Rev. Plant Physiol. 25:135-166.

Murashige, Toshio and Folke Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15(3):473-497.

Murillo R., Mario, Alex Kroneberg, Julio F. Mata, José G. Calzada, and Víctor Castro. 1983. Estudio preliminar sobre factores inhibidores de enzimas proteolíticas en la harina de pejibaye (Bactris gasipaes). Rev. Biol. Trop. 31(2):227-231.

National Research Council. 1975. Pejibaye. Pages 73-77 in Underexploited tropical plants with promising economic value. National Academy Press, Washington, D.C.

Noiret, J. M., J. P. Gascon, and C. Pannetier. 1985. La production de palmier à huile par culture in vitro (Oil palm production through in vitro culture). Oléagineux 40(7):365-370. (English translation).

Nwankwo, B. A., and A. D. Krikorian. 1982. Water as a storage medium for Elaeis guineensis (pisifera) seeds under aseptic conditions. Ann. Bot. 50:793-798.

Nwankwo, B. A., and A. D. Krikorian. 1983. Morphogenetic potential of embryo- and seedling-derived callus of Elaeis guineensis Jacq. var. pisifera Becc. Ann. Bot. 51(1):65-76

Omar, M. S., and M. B. Arif. 1985. An investigation of the fate of Phoenix dactylifera L. carpels cultured in vitro. Date Palm Journal 4(1):15-24.

Opute, F. I., and C. O. Obascola. 1979. Breeding for short-stemmed oil palm in Nigeria: Fatty acids, their significance and characteristics. Ann. Bot. 43:677-681.

Pacheco, A. R., I. M. Barnwell, and B. J. Tailliez. 1986. Des cas de déficience en cuivre en pépinière de palmiers à huile en Amazonie brésilienne (Cases of copper deficiency in oil palm nurseries of Brazilian Amazonia) (French, English summary). Oléagineux 41(11):483-489.

Padmanabhan, D. 1976. Rare axillary shoots in Phoenix. Principes 20:91-97.

Padolina, William G. 1985. Contribution of biological research to the development of the coconut industry. JAOCS (J. Am. Oil Chem. Soc.) 62(2):206-210.

Pannetier, C., and J. Buffard-Morel. 1982. Premiers résultats concernant la production d'embryons somatiques à partir de tissus foliaires de cocotier, Cocos nucifera L. (First results of somatic embryo production from leaf tissue of coconut, Cocos nucifera L.). Oléagineux 37(7):349-354. (English translation).

Pannetier, C., and J. Buffard-Morel. 1983. Production of somatic embryos on leaf tissues of coconut, Cocos nucifera L. Pages 755-756 in Fujiwara, A., ed. Plant tissue culture. Proceedings of the fifth international congress of plant tissue and cell culture, Tokyo, Japan, 1982. Maruzen, Tokyo (distributor).

Pannetier, C., and J. Buffard-Morel. 1985. Coconut palm (Cocos nucifera L.). Pages 430-450 in Bajaj, Y. P. S.,ed. Biotechnology in agriculture and forestry. Vol. 1. Springer-Verlag, Berlin, West Germany.

Pannetier, C., P. Arthuis, and D. Lievoux. 1981. Néoformation de jeunes plantes d'Elaeis guineensis à partir de cals primaires obtenus sur fragments foliaires cultivés in vitro (Neoformation of young Elaeis guineensis plants from primary calluses obtained on leaf fragments in vitro). Oléagineux 36(3):119-122. (English translation).

Paranjothy, K. 1982. A review of tissue culture of oil palm and other palms. Occasional Paper No. 3. PORIM (Palm Oil Res. Inst.), Malaysia.

Paranjothy, K. 1984. Oil Palm. Pages 591-605 in Ammirato, Philip V., David A. Evans, William R. Sharp, and Yasuyuki Yamada, eds. Handbook of plant cell culture. Vol. 3. Macmillan Publishing Company, New York.

Paranjothy, K. 1986. Recent developments in cell and tissue culture of oil bearing palms. PORIM (Palm Oil Res. Inst., Malaysia) Occasional Paper No. 19. 12 pp.

Pasberg-Gauhl, C. 1986. Propagación vegetativa del pejibaye (Bactris gasipaes H.B.K.) por cultivo de tejidos. Centro Agronómico Tropical de Investigación y Enseñanza, CATIE, Turrialba, Costa Rica. 12 pp.

Patiño, Victor Manuel. 1958. El cachipay o pijibay en la cultura de los indígenas de la América intertropical. Ediciones Especiales No. 39. Instituto Indigenista Interamericano, Mexico.

Pérez, V. J. M. 1983. Palmeras de la zona de Yurimaguas, Perú. Pages 165-166 in Palmeras poco utilizadas de América tropical. Reunión de consulta. (Proceedings of the workshop on underutilized palms of tropical America, 1983, Turrialba, Costa Rica). FAO. Vol. 43. FAO/CATIE, San José, Costa Rica.

Pinedo Panduro, Mario Herman. 1987. Organogénesis directa en ápices caulinares de pejibaye (Bactris gasipaes H.B.K.). Master's Thesis. Centro Agronómico Tropical de Investigación y Enseñanza, Turrialba, Costa Rica. 110 pp.

Popenoe, John. 1977. Growing palms with edible fruits in Florida. Proc. Fla. State Hort. Soc. 90:211-212.

Popenoe, Wilson, and Otón Jiménez. 1921. The pejibaye: A neglected food-plant of tropical America. J. Heredity 12:154-166.

Quisumbing, E. 1926. Branching in coconut. Philipp. Agric. 15:3-11.

Rabéchault, H. and S. Cas. 1974. Recherches sur la culture in vitro des embryons de palmier à huile (Elaeis guineensis Jacq. var dura Becc.). X. Culture de segments d'embryons (The in vitro cultures of oil palm embryos, Elaeis guineensis var. dura. X. Culture of embryo segments). Oléagineux 29(2):73-78. (English summary).

Rabéchault, H., and J.-P. Martin. 1976. Multiplication végétative du palmier à huile (Elaeis guineensis Jacq.) à l'aide de cultures de tissus foliaires (Vegetative propagation of oil palm (Elaeis guineensis) by means of leaf tissue culture. C. R. Acad. Sci., Sér. D 283(16):1735-1737.

Rabéchault, H., J. Ahée, and G. Guénin. 1968. Recherches sur la culture in vitro des embryons de palmier à huile (Elaeis guineensis Jacq.). IV. Effets de la teneur en eau des noix et de la durée de leur stockage (Research on the culture in vitro of oil palm embryos (Elaeis guineensis Jacq.). IV. Effect of the water content of the kernels and of the length of storage). Oléagineux 23:233-237. (English summary).

Rabéchault, Henri, Jeanne Ahée, and Gilbert Guénin. 1970. Colonies cellulaires et formes embryoïdes obtenues in vitro à partir de cultures d'embryons de palmier à huile (Elaeis guineensis Jacq. var. dura Becc.). C. R. Acad. Sci. 270:3067-3070.

Rabéchault, H., J. Ahée, and G. Guénin. 1972a. Recherches sur la culture in vitro des embryons de palmier à huile (Elaeis guineensis Jacq. var. Dura Becc.). VIII. Action du lait de coco autoclavé en présence ou non de gélose et de lumière, et en raison de l'âge des graines (The in vitro culture of oil palm embryos, Elaeis guineensis var. dura. VIII. The action of autoclaved coconut milk in the presence or absence of agar and light and in relation to seed age). Oléagineux 27(5):249-254. (English summary).

Rabéchault, H., J. P. Martin, and S. Cas. 1972b. Recherches sur la culture des tissus de palmier à huile (Elaeis guineensis Jacq.) (Research on the culture of oil Palm tissues, Elaeis guineensis Jacq.). Oléagineux 27(11):531-534. (English summary).

Rabéchault, H., G. Guénin, and J. Ahée. 1973. Recherches sur la culture in vitro des embryons de palmier à huile (Elaeis guineensis Jacq. var. Dura Becc.). IX. Activation de la sensibilité au lait de coco par une réhydratation des graines (The in vitro culture of oil palm embryos, Elaeis guineensis var. dura. IX. Activation of the response to cocnut milk by rehydration of seeds). Oléagineux 28(7):333-336. (English summary).

Rabéchault, H., J. Buffard-Morel, and C. Varechon. 1974. Recherches sur la culture in vitro des embryons de palmier à huile (Elaeis guineensis Jacq.). XI. Effets de la pression osmotique sur la croissance et le développement et sur l'absorption des sucres (The in vitro culture of oil palm embryos, Elaeis guineensis. XI. Effects of osmotic pressure on growth and development and on absorbtion of sugars). Oléagineux 29(7):351-356. (English summary).

Rabéchault, H., J. Ahée, and G. Guénin. 1976. Recherches sur la culture in vitro de palmier à huile (Elaeis guineensis Jacq.). XII. Effets de substances de croissance à des doses supraoptimales. Relation avec le brunissement des tissus (The in vitro culture of oil palm embryos (Elaeis guineensis). XII. Effects of supraoptimal doses of growth substances. Relationship with tissue browning). Oléagineux 31(4):159-163.

Radley, M., and E. Dear. 1958. Occurrence of gibberellin-like substances in coconut. Nature 182:1098.

Raju, C. R., K. P. Prakash, M. Chandramohan, and R. D. Iyer. 1984. Coconut plantlets from leaf tissue cultures. J Plant. Crops (Short scientific reports) (India) 12(1):75-78.

Reinert, J. 1970. Control of morphogenesis in plant tissue cultured in vitro. Pages 686-694 in Carr, D. J., ed. Plant growth substances. Springer-Verlag, New York.

Reuveni, O. 1979. Embryogenesis and plantlets growth of date palm (Phoenix dactylifera L.) derived from callus tissue. Plant Physiol. 63(5, Suppl.):138. (Abstr.)

Reuveni, O., and H. Lilien-Kipnis. 1974. Studies on the in vitro culture of date palm (Phoenix dactylifera L.) tissues and organs. Pamphlet No. 145. The Volcani Institute of Agricultural Research, Israel. 42 pp.

Reynolds, J. F. 1981. Asexual embryogenesis in callus derived from palm inflorescences. Page 436 in International symposium on propagation of higher plants through tissue culture: Emerging technologies and strategies. Proceedings, Knoxville, Tennessee, 1980. Environ. Exp. Bot. 21(3-4):436.

Reynolds, John F., and Toshio Murashige. 1979. Asexual embryogenesis in callus cultures of palms. In Vitro 15(5):383-387.

Reynolds, J. F. and T. Murashige. 1982. Vegetative propagation of palm trees. Pages 182-207 in Bonga, J. M. and D. J. Durzan, eds. Tissue culture in forestry. Martinus Nijhoff/Dr. W. Junk, Publishers, The Hague, Holland.

Rhodes, J. M., and L. S. C. Wooltorton. 1978. The biosynthesis of phenolic compounds in wounded plant storage tissues. Pages 243-308 in Kahl, G., ed. Biochemistry of wounded plants. W. de Gruyter & Co., New York, NY.

Roca, W. M. 1983. Cultivo de tejidos de palmas poco conocidas. Pages 45-?? in Reunión de consulta sobre palmeras poco utilizadas de América tropical (Proceedings of the workshop on underutilized palms of tropical America, 1983, Turrialba, Costa Rica). FAO. Vol. 43. FAO/CATIE, San José, Costa Rica.

Roca, William. 1986. Clonaje de células vegetales in vitro. Colombia: Ciencia y Tecnología 4(1):14-15, 29.

Romberger, J. A., and C. A. Tabor. 1971. The Picea abies shoot apical meristem in culture. I. Agar and autoclaving effects. Am. J. Bot. 58(2):131-140.

Rossell, J. B., B. King, and M. J. Downes. Composition of oil. JAOCS (J. Am. Oil Chem. Soc.) 62(2):221-229.

Ruiz, M. E. 1982. Crop-animal production systems research at CATIE. Pages 31-40 in Fitzhugh, H.A., R. D. Hart, R. A. Moreno, P. O. Osuji, M. E. Ruiz, and L. Singh, eds. Research on crop-animal systems. Centro Agronómico Tropical de Investigación y Enseñanza, Caribbean Agricultural Research and Development Institute, and Winrock International, Turrialba, Costa Rica.

Salazar, Sebastián S. 1986. The effect of 2,4-D, light, and activated charcoal on offshoot-tip culture of the pejibaye palm (Bactris gasipaes H.B.K.). Master's Thesis. University of Birmingham, Birmingham, England. 70 pp.

Sattler Z., Rafael. 1985a. Reconocimiento de nuevas fuentes de aceite y grasa a partir de palmas oleaginosas nativas del trópico húmedo americano. CATIE, Turrialba, Costa Rica. 20 pp.

Sattler Z., Rafael. 1985b. Propuesta formal de invetigación. Metodología de trabajo. Propuesta de investigación sobre la propagación vegetativa del pejibaye. (Proposal for research on the vegetative propagation of pejibaye.) CATIE, Turrialba, Costa Rica. 5 pp.

Sattler Z., Rafael. 1985c. Informe de avance (Progress report), 1 de junio a 30 de septiembre, 1985. Proyecto "Reconocimiento de nuevas fuentes de aceite y grasa a partir de palmas oleaginosas nativas del trópico húmedo americano." CATIE, Turrialba, Costa Rica. 4 pp.

Sattler Z., Rafael. 1985d. Informe de avance trimestral (Trimester progress report), 1 de julio a 30 de septiembre, 1985. Proyecto "Reconocimiento de nuevas fuentes de aceite y grasa a partir de palmas oleaginosas del trópico húmedo americano." CATIE, Turrialba, Costa Rica. 4 pp.

Sattler Z., Rafael. 1985e. Informe de avance trimestral (Trimester progress report), 1 de octubre a 31 de diciembre, 1985. Proyecto "Reconocimiento de nuevas fuentes de aceite y grasa a partir de palmas oleaginosas nativas del trópico húmedo americano." CATIE, Turrialba, Costa Rica. 12 pp.

Sattler Z., Rafael. 1986a. Informe de avance (Progress report) del 1 de octubre del 85 al 15 de enero del 86. CATIE, Turrialba, Costa Rica. 4 pp.

Sattler Z., Rafael. 1986b. Informe final (Final Report), 1 de julio, 1985 al 28 de febreo, 1986. Proyecto "Reconocimiento de nuevas fuentes de aceite y grasa a partir de palmas oleaginosas nativas del trópico húmedo americano." CATIE, Turrialba, Costa Rica.

Schroedar, C. A. 1970. Tissue culture of date shoots and seedlings. Date Growers' Inst. Rept. 47:25-27.

Schwabe, W. W. 1984. Scope and possibilities of tissue culture techniques. Pages 11-24 in Micropropagation of selected rootcrops, palms, citrus, and ornamental species. Proc. Symp. on plant tissue culture, technology, and utilization. As, Norway. 1984. FAO Plant Production and Protection Paper No. 59.

Seibert, R. J. 1950. The importance of palms to Latin America; Pejibaye a notable example. Ceiba (Honduras) 1(2):65-74.

Shantz, E. M., and F. C. Steward. 1955. The identification of compound A from coconut milk as 1,3-diphenylurea. J. Am. Chem. Soc. 77:6351-6353.

Sharma, D. R., Rita Kumari, and J. B. Chowdhury. 1980. In vitro culture of female date palm (Phoenix dactylifera L.) tissues. Euphytica 29(1):169-174.

Sharma, D. R., Sunita Dawra, and J. B. Chowdhury. 1984. Somatic embryogenesis and plant regeneration in date palm (Phoenix dactylifera Linn) cv. 'Khadravi' through tissue culture. Indian J. Exp. Biol. 22(11):596-598.

Sharma, D. R., Sunita Deepak, and J. B. Chowdhury. 1986. Regeneration of plantlets from somatic tissues of the date palm Phoenix dactylifera Linn. Ind. J. Exp. Biol. 24:763-766.

Sine, Charlotte, ed. 1987. Farm chemical handbook '87. Meister Publishing Co., Willoughby, Ohio. 561 pp.

Singha, S. 1984. Influence of two commercial agars on in vitro shoot proliferation of 'Almey' crabapple and 'Seckel' pear. HortScience 19:227-228.

Skoog, Folke, and Carlos O. Miller. 1957. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp. Soc. Exp. Biol. 11:118-131.

Smith, B. G. 1984. Tissue culture of Cocos nucifera; Biochemical changes preceding embryogenesis. Unilever Research, Bedford, U. K. 5 pp.

Smith, R. W., and J. M. S. Allen. 1981. Revitalising the 'tree of life': The future for coconuts. Span 24:118-120.

Smith, S. N. 1975. Vegetative propagation of the date palm by root tip culture. Bull. Agron. Sahariènne 1(3):67.

Smith, W. K., and J. A. Thomas. 1973. The isolation and in vitro cultivation of cells of Elaeis guineensis. Oléagineux 28(3):123-127.

Snedecor, George W., and William G. Cochran. 1956. Statistical methods. Fifth edition. The Iowa State College Press, Ames, Iowa. 534 pp.

Soh, A. C. 1986. Expected yield increase with selected oil palm clones from current DxP seedling materials and its implications on clonal propagation, breeding and ortet selection. Oléagineux 41(2):51-56.

Srinivasan, C., R. E. Litz, J. Barker, and Knut Norstog. 1985. Somatic embryogenesis and plantlet formation from Christmas palm callus. HortScience 20(2):278-280.

Staritsky, G. 1970. Tissue culture of the oil palm (Elaeis guineensis Jacq.) as a tool for its vegetative propagation. Euphytica 19(3):288-292.

Staritsky, G., and C. J. Breure. 1985. The morphogenesis of the inflorescence of the oil palm in relation to yield components. Acta Bot. Neerl. 34(4):437-438. (Abstr.)

Staritsky, G., and E. A. Zandvoort. 1985. In vitro propagation and genetic conservation of tropical woody crops. Acta Bot. Neerl. 32(2):238. (Abstr.)

Stoltz, Leonard P. 1971. Agar restriction of the growth of excised mature iris embryos. J. Am. Soc. Hort. Sci. 96(5):681-684.

Sudasrip, H. 1980. Reversal of Arenga pinnata spadices into vegetative shoots and its relevance to the origin of coconut bulbils. Principes 24(4):147-153.

Thanh-Tuyen, Nguyen T., and Emerita V. de Guzman. 1983. Pollen development stages for coconut anther culture. Kalikasan, Philipp. J. Biol. 12(1-2):135-144.

Thomas, Donovan des S., and Toshio Murashige. 1979a. Volatile emissions of plant tissue cultures. I. Identification of the major components. In Vitro 15(9):654-658.

Thomas, Donovan des S., and Toshio Murashige. 1979b. Volatile emissions of plant tissue cultures. II. Effects of the auxin 2,4-D on production of volatiles in callus cultures. In Vitro 15(9):659-663.

Thomas, R. L., A. K. Seth, K. W. Chan, and S. C. Ooi. 1973. Induced parthenocarpy in the oil-palm. Ann. Bot. 37(151):447-452.

Thomas, V., and P. S. Rao. 1985. In vitro propagation of oil palm (Elaeis guineensis Jacq var Tenera) through somatic embryogenesis in leaf-derived callus. Current Science 54(4):184-185.

Thorpe, Trevor A., and K. R. Patel. 1984. Clonal propagation: Adventitiuos buds. Pages 49-60 in Vasil, Indra K., ed. Cell culture and somatic cell genetics of plants. Vol. 1. Academic Press, New York.

Tisserat, Brent. 1979a. Propagation of date palm (Phoenix dactylifera L.) in vitro. J. Exp. Bot. 30(119):1275-1283.

Tisserat, Brent. 1979b. Tissue culture of the date palm. J. Her. 70(3):221-222.

Tisserat, Brent. 1981. Date palm tissue culture. U.S. Department of Agriculture. Agricultural Research Service. Advances in Agricultural Technology. Western Series, No. 17. (U.S. Department of Agriculture, Oakland, Calif.). 50 pp.

Tisserat, Brent. 1982. Factors involved in the production of plantlets from date palm callus cultures. Euphytica 31(1):201-214.

Tisserat, Brent. 1983a. Asexual embryogenesis in palms. In Vitro 19(3):237. Summary.

Tisserat, Brent. 1983b. Development of new tissue culture technology to aid in the cultivation and crop improvement of date palms. Pages 126-140 in Proceedings of the first symposium on the date palm in Saudi Arabia, King Faisal University, 1982. Al-Hassa, Saudi Arabia.

Tisserat, Brent. 1983c. Tissue culture of date palms-a new method to propagate an ancient crop-and a short discussion of the California date industry. Principes 27(3):105-117.

Tisserat, Brent. 1984a. Clonal propagation: Palms. Pages 74-81 in Vasil, Indra K., ed. Cell culture and somatic cell genetics of plants. Vol.1. Academic Press, Inc., New York.

Tisserat, B. 1984b. Date Palm. Pages 505-545 in Sharp, William R., David A. Evans, Philip V. Ammirato, and Yasuyuki Yamada, eds. Handbook of Plant Cell Culture. Vol. 2. Macmillan Publishing Company, New York.

Tisserat, Brent. 1984c. Propagation of date palms by shoot tip cultures. HortScience 19(2):230-231.

Tisserat, B., and D. A. DeMason. 1980. A histological study of development of adventive embryos in organ cultures of Phoenix dactylifera L. Ann. Bot. 46(4):465-472.

Tisserat, Brent, and Darleen A. DeMason. 1985. Occurrence and histological structure of offshoots and inflorescences produced from Phoenix dactylifera L. plantlets in vitro. Bull. Torrey Bot. Club 112(1):35-42.

Tisserat, Brent, Jene M. Ulrich, and Bernard J. Finkle. 1981. Cryogenic preservation and regeneration of date palm tissue. HortScience 16(1):47-48.

Tomlinson, P. B. 1961. Palmae. Vol 2. of Metcalfe, C. R., ed. Anatomy of monocotyledons. Oxford University Press, Clarendon, England. 453 pp.

Torres, A. M., and B. Tisserat. 1980. Leaf isozymes as genetic markers in date palms. Am. J. Bot. 67:162-167.

Tracy, Mitchell Dean. 1985. The pejibaye fruit: Problems and prospects for its development in Costa Rica. Master's Thesis. The University of Texas at Austin. 111 pp.

Tracy, Mitchell. 1986. Processing and some potential uses of pejibaye (Bactris gasipaes H.B.K.) meal. Interciencia 11(4):173-177.

Tulecke, W., L. H. Weinstein, A. Rutner, and H. J. Laurencot. 1961. The biochemical composition of coconut water (coconut milk) as related to its use in plant tissue culture. Contrib. Boyce Thompson Inst. 21:115-128.

Turnham, Elaine, and D. H. Northcote. 1982. The use of acetyl-CoA carboxylase activity and changes in wall composition as measures of embryogenesis in tissue cultures of oil palm (Elaeis guineensis). Biochem. J. 208(2):323-332.

Turnham, Elaine and D. H. Northcote. 1984. The incorporation of [1-^{14}C]acetate into lipids during embryogenesis in oil palm tissue cultures. Phytochemistry 23(1):35-39.

Ulrich, Jane M., Bernard J. Finkle, and Brent H. Tisserat. Effects of cryogenic treatment on plantlet production from frozen and unfrozen date palm. Plant Physiol. 69(3):624-627.

Valverde, Roberto, and Oscar Arias. 1986a. Efecto de la luz sobre la morfogénesis de tejidos de pejibaye cultivados in vitro. Pages 356-358 in VII Congreso Agronómico Nacional, XXXIII Congreso de Horticultura (A.S.H.S.-Región Tropical). Vol. 1. Colegio de Ingenieros Agrónomos de Costa Rica and Sociedad Americana de Ciencias Hortícolas Región Tropical (A.S.H.S.), San José, Costa Rica. (Abstr.)

Valverde, Roberto, and Oscar Arias. 1986b. Efecto del picloram sobre la morfogénesis del pejibaye (Bactris gasipaes H.B.K.) in vitro. Pages 358-359 in VII Congreso Agronómico Nacional, XXXIII Congreso de Horticultura (A.S.H.S.-Región Tropical), vol 1. Colegio de Ingenieros Agrónomos de Costa Rica and Sociedad Americana de Ciencias Hortícolas Región Tropical (A.S.H.S.), San José, Costa Rica. (Abstr.)

Valverde, Roberto, Òscar Arias, and Trevor A. Thorpe. 1987. Picloram-induced somatic embryogenesis in pejibaye palm (Bactris gasipaes H.B.K.). Plant Cell, Tissue and Organ Culture 10:149-156.

Vandermeer, John. 1977. Observations on the root system of the pejibaye palm (Bactris gasipaes H.B.K.) in Costa Rica. Turrialba 27(3):239-242.

Vandermeer, J. 1983. Pejibaye palm. Pages 98-101 in Janzen, Daniel H., ed. Costa Rican natural history. The University of Chicago Press, Chicago, Illinois.

van Staden, J. 1974. Evidence for the presence of cytokinins in malt and yeast extracts. Physiol. Plant. 30(2):182-184.

van Staden, J., and S. E. Drewes. 1974. Identification of cell division inducing compounds from coconut milk. Physiol. Plant. 32(4):347-352.

van Staden, J., and S. E. Drewes. 1975. Identification of zeatin and zeatin riboside in coconut milk. Physiol. Plant. 34:106-109.

Vargas, E., and M. Villaplana. 1979. Principales enfermedades del pejibaye observadas en Costa Rica. ASBANA 3(7):8-9.

Vasil, Indra K., ed. 1986. Cell culture and somatic cell genetics of plants. Vol. 3. Academic Press, Inc., New York. 657 pp.

Vavilov, N. I. 1951. The origin, variation, immunity and breeding of cultivated plants. Chronica Botanica 13:1-364.

Villiers, T. A. 1972. Ageing and longevity of seeds in field conditions. Pages 265-288 in Heydecker, W., ed. Seed ecology. Butterworths, London, England.

Von Arnold, S., and T. Ericksson. 1984. Effect of agar concentration on growth and anatomy of adventitious shoots of Picea abies (L.) Karst. P. C. T. O. Culture 3:257-264.

Wallace, Alfred Russel. 1971. Palm Trees of the Amazon. A fascimile reprint of the original edition of 1853. Coronado Press, Lawrence, Kansas. 129 pp.

Wang, P.-J., and L.-C. Huang. 1976. Beneficial effects of activated charcoal on plant tissue and organ cultures. In Vitro 12:260-262.

Warley, A., D. Ferdinando, and W. A. Hughes. 1985. Application of X-ray microanalysis to cell suspensions of oil palm (Elaeis guineensis Jacq.). Planta 165:527-531.

Weatherhead, M. A., J. Burdon, and G. G. Henshaw. 1978. Some effects of activated charcoal as an additive to plant tissue culture media. Z. Pflanzenphysiol. 89(2):141-147.

Weatherhead, M. A., J. Burdon, and G. G. Henshaw. 1979. Effects of activated charcoal as an additive plant tissue culture media: Part 2. Z. Pflanzenphysiol. 94(5):399-405.

Weiss, Julian M. 1987. Malaysia and US slipping and sliding over oil palm exports. The Christian Science Monitor, Friday, November 20, 1987, page 22.

Wiest, Ray E. 1963. Motivational factors influencing selection in the propagation of the pejibaye palm. An anthropological study of plant selection in Costa Rica. Department of Anthropology, University of Oregon at Corvallis. 18 pp. Unpublished.

Winton, L., and O. Huhtinen. 1976. Tissue culture of trees. Pages 243-264 in Miksche, J. P., ed. Modern methods in forest genetics. Springer-Verlag, Berlin, West Germany.

Wooi, Kheng Choo. 1984. Palm tissue culture. Pages 88-112 in Micropropagation of selected rootcrops, palms, citrus, and ornamental species. Proc. Symp. on plant tissue culture, technology, and utilization. As, Norway, 1984. FAO Plant Production and Protection Paper No. 59.

Wooi, K. C., C. Y. Wong, and R. H. V. Corley. 1982a. Genetic stability of oil palm callus cultures. Pages 749-750 in Fukjiwara, A., ed. Plant tissue culture 1982. Proc. 5th Int. Congr. on plant tissue and cell culture. Maruzen Company, Tokyo.

Wooi, Kheng Choo, Choong Yew Wong, and R. H. V. Corley. 1982b. Tissue culture of palms-A review. Pages 138-144 in Rao, A. N., ed. Tissue culture of economically important plants. Proc. Int. Symp., National University of Singapore, Singapore, 1981. The Committee on Science and Technology in Developing Countries (COSTED) and Asian Network for Biological Sciences (SIBiol), Singapore.

Zaid, A., and B. Tisserat. 1983a. In vitro shoot tip differentiation in Phoenix dactylifera L. Date Palm J. 2(2):163-182.

Zaid, Abdelouahbad, and B. Tisserat. 1983b. Morphogenetic responses obtained from a variety of somatic explant tissues of date palm Phoenix dactylifera. Bot. Mag. 96(1042):67-73.

Zaid, Abdelouahhab, and Brent Tisserat. 1984. Survey of the morphogenetic potential of excised palm embryos *in vitro*. Crop Res. 24(1):1-9.

Zapata, Angel. 1971. Pejibaye palm from the pacific coast of Colombia (a detailed chemical analysis). Econ. Bot. 26:156-158.

Zumbado, Mario E., and Mario G. Murillo. 1984. Composition and nutritive value of pejibaye (*Bactris gasipaes*) in animal feeds. Rev. Biol. Trop. 32(1):51-56.

APPENDIX A: ILLUSTRATIONS OF THE PEJIBAYE PALM

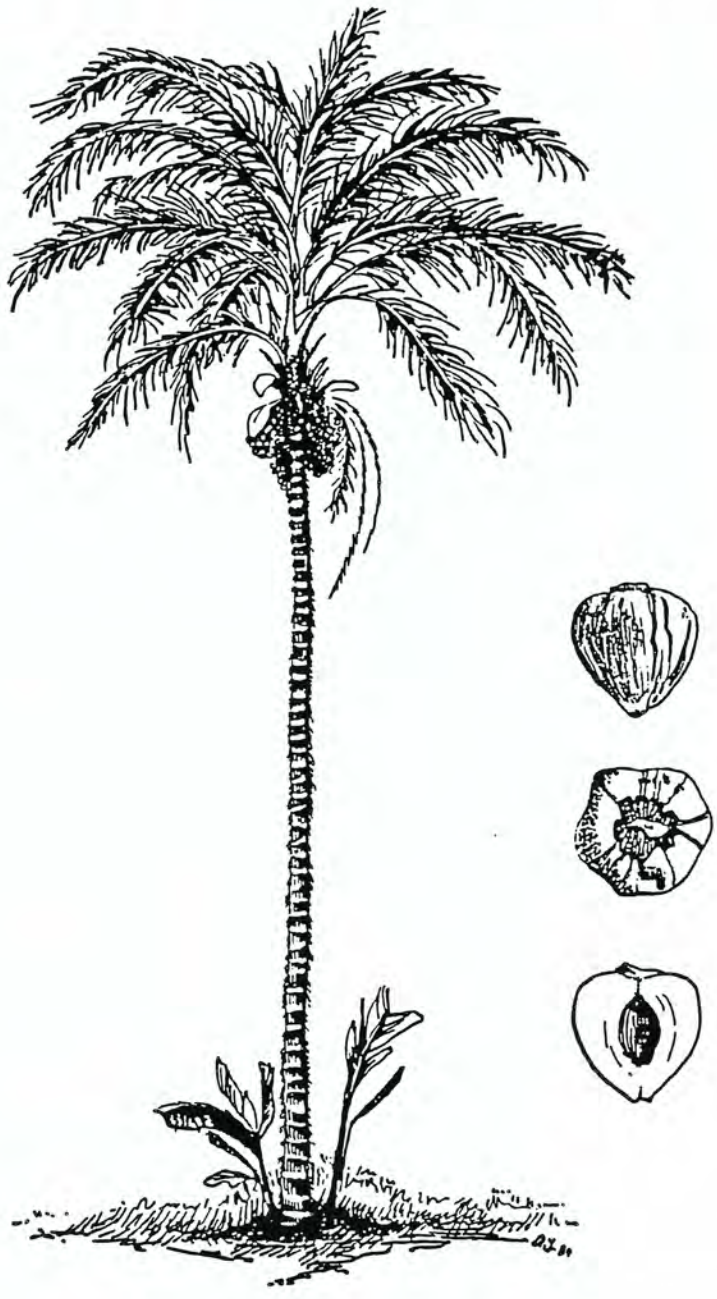


Figure A-1. Drawing of the adult pejibaye palm showing basal offshoots, a straight trunk with spiny internodes, two fruit racemes, and feathery leaves. Drawings of the sideview, topview, and longitudinal section of the fruit are included (not drawn to scale). (Mora-Urpí et al., 1984. Used with permission of the author.)

Figure A-2. Photograph of a ripe fruit bunch of the pejibaye palm (Mora-Urpí et al., 1984. Used with permission of the author.)

The Apical Meristem

The apical meristem of palms lies in the center of the apex of the stem, and not at the apex of a terminal cone, as is the case with other plants (Tomlinson, 1961). For seedlings such as the ones used in this study, which did not exceed a size of 60 cm (measured from the broad stem base to the tallest leaf tip), it means that the shoot tip containing the apical meristem lies no more than 1.5 cm above the root zone, because at the early growth stages of palms the stem is compressed near the root zone until internodal elongation sets in, which usually does not occur until the stem has virtually reached its maximum thickness. The apical meristem is encapsuled within the tubular bases of leaf primordia which "increase in diameter to keep pace with the increase in diameter of the nodes on which they are inserted" (Tomlinson, 1961). The apical meristem in palms is comparable in size to that of other angiosperms. It is largely a leaf-producing meristem and contributes little to stem development.

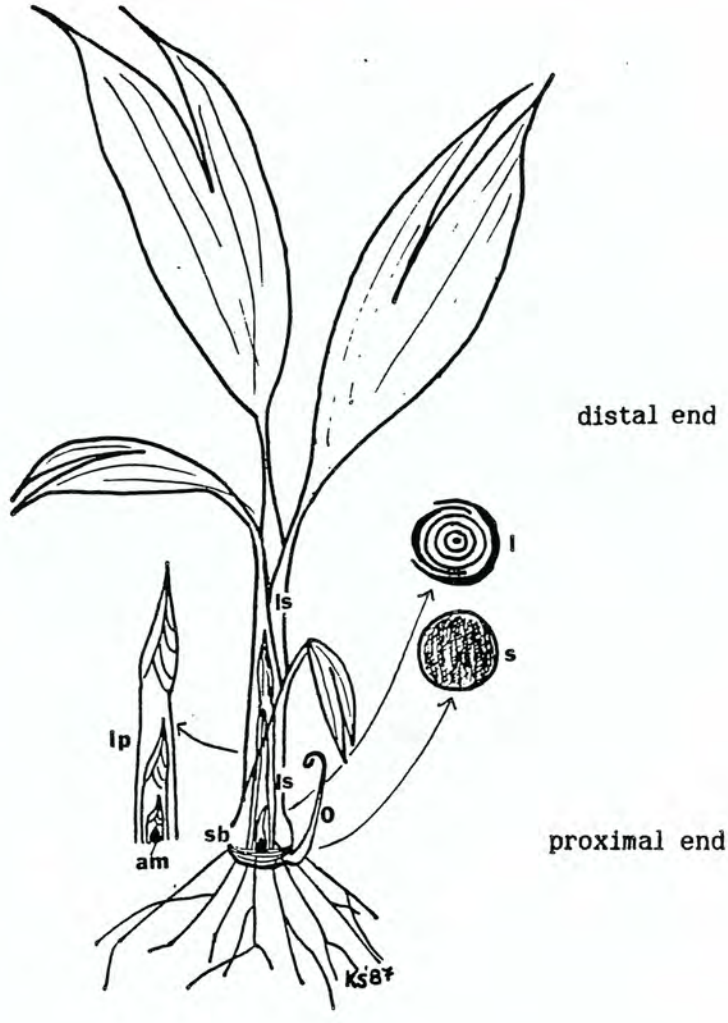


Figure A-3. Sketch of a pejibaye palm seedling showing broad stem base (sb); one offshoot (o) starting to develop; arrangement of leaf primordia (lp) within the leaf-sheaths (ls); position of the apical meristem (am); and arrows indicating the area from which subapical stem sections (s) and leaf-sheath sections (l) were extracted

Figure A-4. A spiny-stemmed and a smooth-stemmed pejibaye seedling, similar to the seedlings dissected for the experiments described in this thesis. Both of these seedlings are 4 months old

APPENDIX B: MISCELLANEOUS OBSERVATIONS

- On several occasions, media solidified with 2 g/l Gelrite liquefied if the cultures had not been subcultured for at least two months. No bacterial contamination was apparent in such cases. This phenomenon underscores the importance of frequent subculturing.

- Pejibaye shoot tips grown on a Murashige and Skoog (1962) medium with 10 mg/l NAA and 2.5 g/l activated charcoal, produced roots and shoot elongation in 40-50 % of the cultures (Fig. B-1). Similar cultures containing BA and coconut water did not induce rooting.

- Stem slices of pejibaye offshoots taken from the field in Costa Rica and cultured on media with high cytokinin:auxin ratios similar to those in PART III, did not grow or show any callus production or organogenesis. The diameter of these slices was 5-6 cm, with a thickness of 5 mm, and they were cultured in disposable 15-cm petri dishes. When opening the petri dishes for subculturing, a vinegar-like odor was released, which was probably a sign of decomposition of the explants. The lack of response in these cultures was attributed to the size of the explants. The use of much smaller and thinner explants, in the order of 1 cm in diameter and 2 mm in thickness, and the use of larger culture vessels are advised for future studies.

- Fungal contamination was usually visible within five days from the establishment of the cultures, whereas bacterial contamination sometimes appeared as late as two months after cultures were established. Such delayed bacterial contamination usually had its origin on the explant and not elsewhere in the culture dish, which suggests that the bacteria were endogenous to the plant tissue.

- The formation of small "tufts" of white callus on vascular bundles was observed on a few, random leaf-sheath cultures (Fig. B-2).

- Leaf-sheath explants that were less than 1 mm thick usually died in culture without growing or producing calli.

- Young leaf tissue, which was sometimes contained in leaf-sheath explants, thickened and elongated on all culture media on which leaf-sheath tissue was grown, but no callus formation was ever observed.

- Ten of the 1000 or so seedlings of the mixed seed lot imported from Costa Rica for this study had variegated leaves. All of the variegated plants were smooth-stemmed. They grew much less vigorously than non-variegated seedlings under greenhouse conditions. The potential of variegated pejibaye palms as indoor ornamental plants should be evaluated.

- In most instances of medium discoloration, the medium turned light brown. In a few cases, however, discoloration was so strong that the medium turned almost black (Fig. B-3).

- Even though no significant differences in browning were observed between shoot tips and leaf-sheath explants in Experiment 2, PART I, there was a trend for shoot tips to exhibit lower browning rates, as illustrated in Fig. B-4. Other experiments supported this trend, such as the shoot tip cultures in PART IV, in which browning was not problematic, versus the leaf-sheath and stem cultures in PART II and PART III, which showed high browning levels. It is possible that the 5 replications in Experiment 2, PART I were not sufficient to detect a significant difference, and further research is needed before it can be stated that the observed trend is only coincidental.

- Culture contamination was low when compared to other reports of contamination in pejibaye tissue culture. It reached 28 % in subapical stem tissue (see PART III), but in shoot-tip cultures it never exceeded 10.5 % (see PART IV). Pinedo Panduro (1987), on the other hand, reported up to 94 % contamination in shoot tips obtained from seedlings, and Pasberg-Gauhl (1986) reported 87 %.

Figure B-1. A pejibaye plantlet that developed from a shoot tip grown on an MS (1962) medium with 10 mg/l NAA and 2.5 g/l activated charcoal. The root (r) developed after 6 weeks in culture

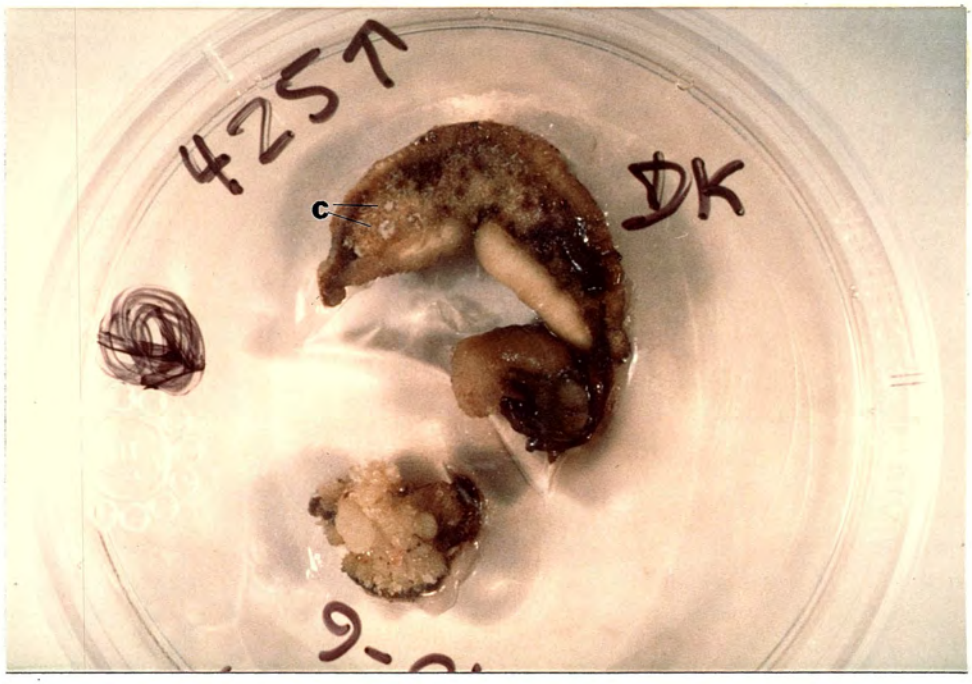
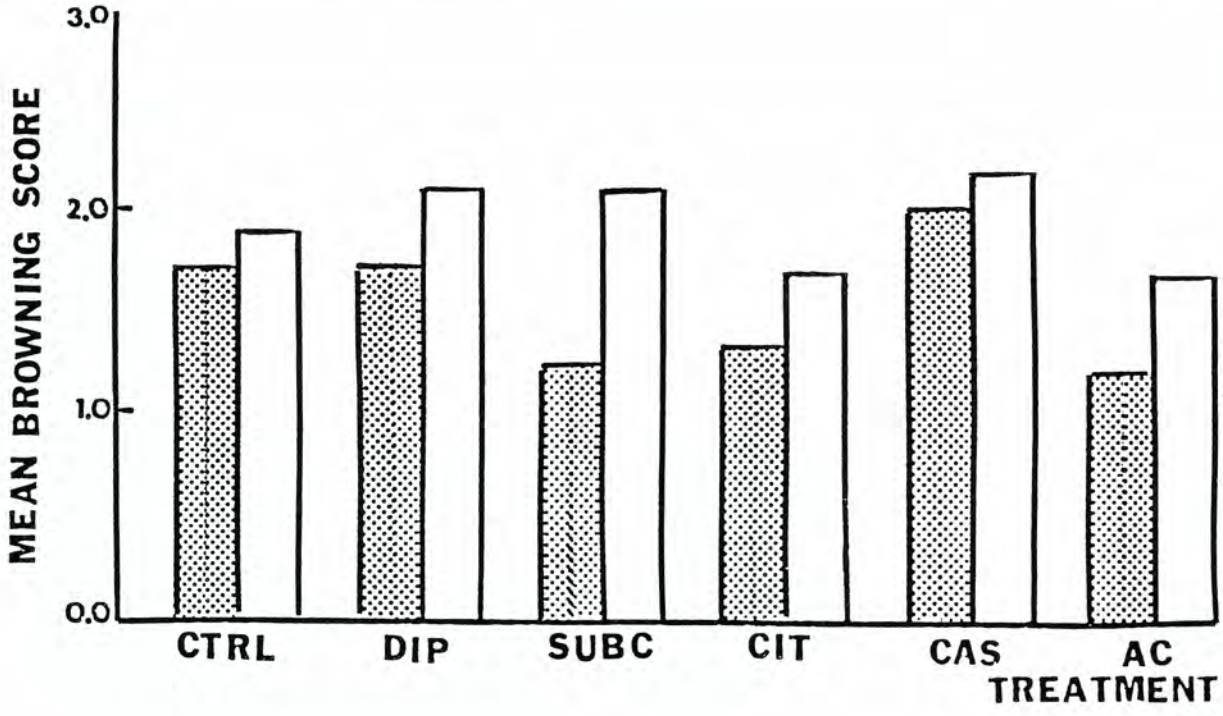


Figure B-2. Small callus "tufts" (c) on vascular bundles of a leaf-sheath explant

Figure B-3. A case of severe medium discoloration. The discolored medium contains a stem callus, the clear medium a shoot-tip callus. Both cultures were 1 month old when the picture was taken, and both were grown on the same medium



= Shoot tips

= leaf-sheath sections

CTRL = Control
DIP = Dip in citric acid and ascorbic acid solution
SUBC = Subculture once a week
CIT = Citric acid added to medium
CAS = Casein hydrolysate added to medium
AC = Activated charcoal added to medium

Figure B-4. Differences in browning means between pejibaye shoot tips and leaf-sheath sections. (Browning scores were assigned as follows: 0 for no browning; 1 for mild browning on the bottom and along the edges of the explant; 2 for dark browning on the bottom, along the edges, and on parts of the upper cut surface of the explant; and 3 for complete browning.)

APPENDIX C: RECOMMENDATIONS FOR FUTURE RESEARCH

- Continue researching the in vitro behavior of stem explants. In those cases where root growth is observed, shoot induction should be attempted.

- Study the in vitro processes of pejibaye tissue culture at a physiological and biochemical level in order to improve the understanding of callus formation, embryogenesis, organogenesis, and browning.

- Continue experimentation not only with growth regulators, but also with other tissue culture parameters such as incubation temperature, sucrose concentration, vitamin additions, macro- and microelement concentrations, and the addition of elements not usually added to tissue culture media as mineral nutrients. Previous research indicates that the adaptation of a general medium formulation, such as Murashige and Skoog's (1962), to the tissue culture of a specific palm species, is important (Chaverri Jiménez, 1984, and Eeuwens, 1976 and 1978).

- Carry out experiments with suspension cultures, protoplast fusion, and anther cultures.

- Study the longevity and viability of shoot-tip calli under long-term storage conditions, including cryogenic storage. The same studies should be performed on other types of pejibaye calli, once producing them becomes a reliable process.